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THE ECOLOGY OF THE LOUSE *POLYPLAX SERRATA* (BURM.) ON THE MOUSE *MUS MUSCULUS* L.

By M. D. MURRAY*

[*Manuscript received June* 30, 1960]

Summary

The number of *Polyplax serrata* (Burm.) on the mouse *Mus musculus* L. is determined by the efficiency with which the mouse grooms itself with its mouth. The efficiency with which the accessible hindpart of the body is groomed is such that normally the majority of all stages of the life cycle are found on the forepart of the mouse with the exception of the stage I nymph which is distributed over the whole body.

The principal requirements for self-grooming to control the number of lice are that the technique is efficient, that sufficient time is spent grooming, that an adequate area of the body is groomed, and that lice move readily into the accessible area. Any factor which influences adversely any one of these requirements causes the efficiency of grooming to decrease, and thus permits lice to increase in numbers and to populate the whole body.

I. INTRODUCTION

The distribution of lice on some species of rodents has been described by Dubinin (1953), who found that on small rodents such as mice and voles it was restricted, usually to the forepart of the body and the root of the tail, but on rats the lice were dispersed over the dorsal and lateral aspects of the body. Vysotskaya (1950) reported that there was a seasonal change in the distribution of the louse *Hoplopleura acanthopus* (Burm.) on the body of the vole, *Microtus arvalis* (Pall.); the lice were found only on the forepart of the body in the autumn but were present over the whole body in early spring. In addition, the lice were found to be most abundant in the late winter and spring. A similar variation in the number of this louse has been found to occur on the male meadow vole *M. pennsylvanicus* (Ord) (Cook and Beer 1958). Seasonal variations in the number of lice on rodents have been reported to occur also on the rats *Rattus norvegicus* Berkenhout (Harkema 1936; Zakovich 1946), *R. rattoides turkestanicus* (Satunin), and *Nesokia indica* (Gray & Hardwicke) (Dubinin 1950); the squirrels *Sciurus carolinensis* Gmelin (Harkema 1936) and *S. vulgaris mantchuricus* Thomas (Dubinin 1950); and on three subspecies of the vole *M. socialis* (Pall.) (Kirshenblat 1938; Olsuf'ev 1940). However, an absence of significant seasonal variations in numbers has been reported on female meadow voles, *M. pennsylvanicus*, and on the deer mouse, *Peromyscus maniculatus bairdii* (Hoy & Kennicott) (Cook and Beer 1958). Thus, variations both in the abundance of the lice on rodents and in the extent of their distribution over their host's body have been recorded.

This paper reports the results of a study of the factors which govern the abundance and distribution of the louse, *Polyplax serrata* (Burm.), on the mouse, *Mus musculus* L.

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II. Life Cycle of P. serrata

(*a*) *General*

P. serrata is a louse of the order Anoplura, family Hoplopleuridae, a family of blood-sucking lice whose members are found mainly on rodents. The five stages of the life cycle are the egg, three nymphal stages, and the adult. The nymphal stages can be readily differentiated, both by size and by the distribution of the setae on the abdomen (Fig. 1). The male and female adults differ markedly in size, the length of the female being approximately one and a half times that of the male. The eggs are attached to the hair near the skin with the pole of attachment nearest to the skin.

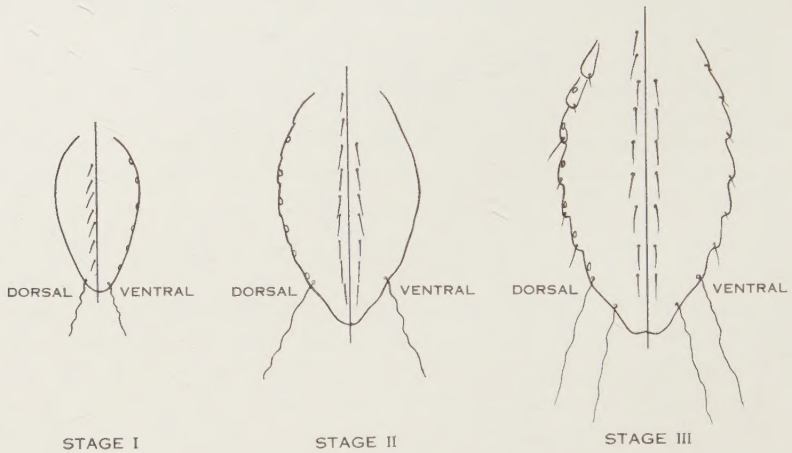

Fig. 1.—Distribution of setae on dorsal and ventral aspects of the abdomen of the nymphs of *Polyplax serrata*.

(*b*) *Length of Life Cycle*

Hairs, with attached eggs, were removed from a mouse and exposed to 35°C, which is within the range of the temperature gradient near the skin of mice (Murray, unpublished data). The humidity was controlled with a saturated sodium dichromate solution to about 54% R.H. (Solomon 1951). A daily examination was made of the 58 eggs exposed and all had hatched by the fifth day.

Stage I nymphs, which had hatched from eggs incubated in the laboratory, were placed on louse-free mice which were fitted with Elizabethan collars round their necks to prevent grooming (Plate 1, Fig. 1). When 25 stage I nymphs were placed on a mouse and 40 on another and the mice killed and examined 7 days later, only adults were found, 10 males and 1 female on one and 7 males and 21 females on the other. About 80 stage I nymphs were placed on a third mouse, and only adult lice, 11 females and 4 males, were found 7 days later. One of the females laid an egg whilst being examined and a fully developed egg was present in the abdomen of each of five others. Approximately 80 stage I nymphs were placed on the head of a fourth mouse which was examined 6 days later when only seven females were found, and a fully developed egg was found within the abdomen of five of them.

Therefore, as the eggs of *P. serrata* can hatch within 6 days and stage I nymphs can develop into adults within 7 days, the life cycle of *P. serrata* can be completed in 13 days.

III. Normal Number and Distribution of P. serrata on the Mouse

(*a*) *Methods*

The hair from the skins of four mice was examined after treatment with xylol (Murray 1957), to determine whether dead lice were retained in the hair coat, but only living lice were found. Hatched and dead eggs, however, remained attached to the hair but these could be readily differentiated from living eggs in which an embryo of normal appearance was visible. As dead lice were not retained

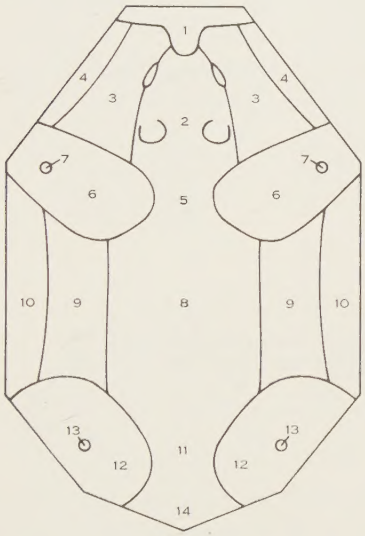

Fig. 2

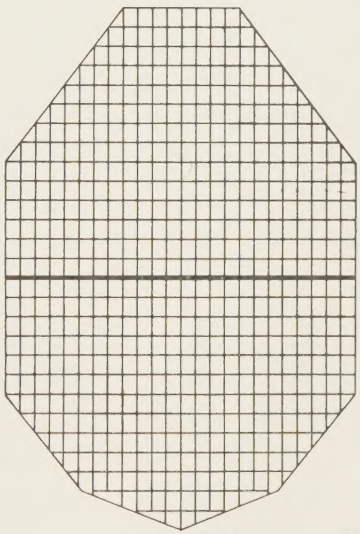

Fig. 3

Fig. 2.—Constant shape to which the skins of the mice were stretched: *1*, nose and around mouth; *2*, forehead, poll; *3*, cheeks, side of neck; *4*, ventral neck; *5*, between shoulder blades; *6*, shoulders, chest; *7*, fore legs; *8*, back; *9*, sides; *10*, belly; *11*, lumbar region; *12*, rump, flank; *13*, hind legs; *14*, root of tail.

Fig. 3.—Grid containing 524 areas. Heavy line denotes line of division into fore- and hindparts.

in the hair coat and dead and living eggs could be differentiated, the number and distribution of all the stages of *P. serrata*, which were living on a mouse when it was killed, could be determined by the following method.

Mice were stunned and their coats were soaked with ether or chloroform to kill all the lice *in situ*. The mice were then placed in a sealed jar together with ether or chloroform until they were dead. Their skins were removed in a similar manner and stretched to conform to a constant shape (Fig. 2), within which had been drawn a grid of 524 units (Fig. 3). All the units, with the exception of 10, were of equal area. In Figure 3 will be seen also a line drawn to divide the body

into fore- and hindparts which were approximately equal in area. The markings of the grid were clearly visible through the skin when it was dry. The hair was dry-shaved from over each area and mounted on a slide in Berlese's mounting medium. Separate preparations were made of the hair from each leg, but the skin on the ears, feet, and tail was examined directly with a stereoscopic microscope.

When it was desired to determine only the number of lice, the skin was stretched over the grid and examined directly with a stereoscopic microscope or the hair was shaved off the skin and examined in xylol.

TABLE 1

DISTRIBUTION AND NUMBER OF EGGS OF POLYPLAX SERRATA ON MICE

Mice N_1, N_2, N_5, N_6, and N_7 were permitted to groom themselves whereas mice C_4, G_1, and G_2 were prevented from grooming by means of an Elizabethan collar

Mouse	Forepart of Body	Hindpart of Body
N_1	299	0
N_2	489	0
N_5	59	0
N_6	356	14
N_7	382	4
C_4	4307	2088
G_1	Between 2500 and 2600	Between 2000 and 2100
G_2	Between 500 and 550	Between 150 and 200

(*b*) *Results*

(i) *Number of Lice.*—A complete count was made of the lice on mice and the average number per mouse was 118·8 (S.D. 49·4; range 36–270). In addition the composition of the louse population on each of four of these mice was determined. There were many more eggs present than lice and more stage I nymphs than the other nymphal or adult stages of the life cycle (Tables 1 and 2).

(ii) *Distribution of Lice.*—All stages, with the exception of stage I nymphs which were scattered over the whole body, were found predominantly on the forepart (Tables 1 and 2). Figure 4 shows where lice were found on mouse N_2, and the predominance of eggs, adults, and stage III nymphs on the forepart of the body and the scattered distribution of stage I nymphs may be seen. Stage II nymphs were more scattered on mouse N_2 than on the other mice. However, of the four nymphs found on the hindpart of the body, two were on the anterior region of that part. Eggs were found mainly around the eyes and ears and between the shoulder blades.

IV. EXPERIMENTAL

It was observed that mice employed two types of self-grooming. They scratched themselves with their hind feet and then licked the toes, and they swept

their mouth through the hair with an upward motion of the head. The restriction of lice normally to the forepart of the body suggested that lice were removed efficiently by grooming only from regions which could be reached by the mouth.

(*a*) *Effect of Prevention of Self-grooming with the Mouth*

To prevent self-grooming of the hindpart of the body with the mouth, Elizabethan collars made of copper wire (Plate 1, Fig. 1) were placed around the

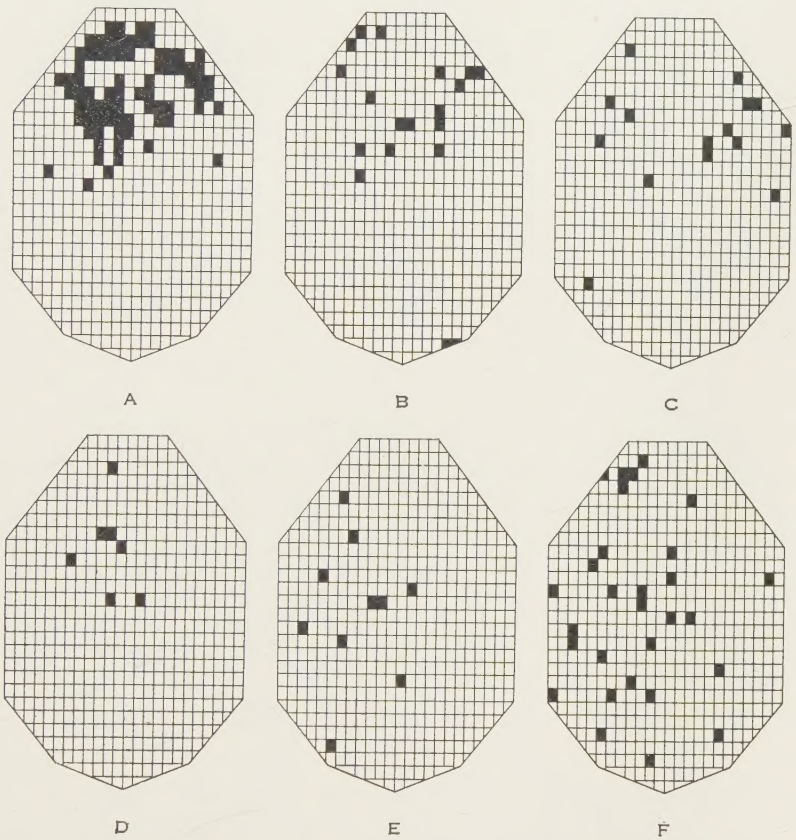

Fig. 4.—Normal distribution of *P. serrata* on the mouse. The black areas are those in which one or more eggs or lice were found. *A*, eggs; *B*, females; *C*, males; *D*, stage III nymphs; *E*, stage II nymphs; *F*, stage I nymphs.

necks of seven mice with normal infestations. The collars did not interfere with their ability to feed and to drink, provided the food and water were placed on the floor of the cage. The mice were killed 3–4 weeks later and the number and distribution of the lice were determined.

The number of lice on one mouse had increased to 868, on five to between 2000 and 4000, and on another to as many as 12,650. The composition of the

TABLE 2

DISTRIBUTION AND NUMBER OF NYMPHS AND ADULT POLYPLAX SERRATA ON MICE

Mice N_1, N_2, N_3, and N_4 were permitted and mice C_1, C_2, C_3, and C_4 were not permitted to groom themselves. Mice E_1 and E_2 were allowed to groom themselves for 24 hr only, after their louse populations had been made to increase by preventing self-grooming

Mouse	Forepart of Body					Hindpart of Body					Whole Body					Total All Stages
	Adults		Nymphs			Adults		Nymphs			Adults		Nymphs			
	♂	♀	III	II	I	♂	♀	III	II	I	♂	♀	III	II	I	
N_1	21	15	10	5	29	1	1	0	1	8	22	16	10	6	37	91
N_2	15	17	9	6	21	2	1	0	4	11	17	18	9	10	32	86
N_3	8	3	5	5	4	0	0	0	0	11	8	3	5	5	15	36
N_4	11	15	15	13	12	0	3	2	2	16	11	18	17	15	28	89
C_1	1413	1485	737	470	595	1949	3138	980	754	1129	3362	4623	1717	1224	1724	12,650
C_2	477	422	306	444	665	347	509	245	327	356	824	931	551	771	1021	4,098
C_3	120	116	55	68	79	60	92	87	74	117	180	208	142	142	196	868
C_4	573			576		532			646		1105			1222		2,327
E_1	559	365	176	135	165	123	206	79	77	131	682	571	255	212	296	2,016
E_2	361	270	116	123	107	73	110	43	46	140	434	380	159	169	247	1,389

louse populations on four of these mice was determined and Tables 1 and 2 show that the number of each stage had increased. Furthermore, numerous lice of all stages were found on the hindpart of the body (Tables 1 and 2; Fig. 5).

Elizabethan collars were placed around the necks of three mice with normal infestations. They were kept apart and weighed regularly for 3 weeks when they were killed. The number of lice on two mice (G_1, G_2) had increased to 1000–1250

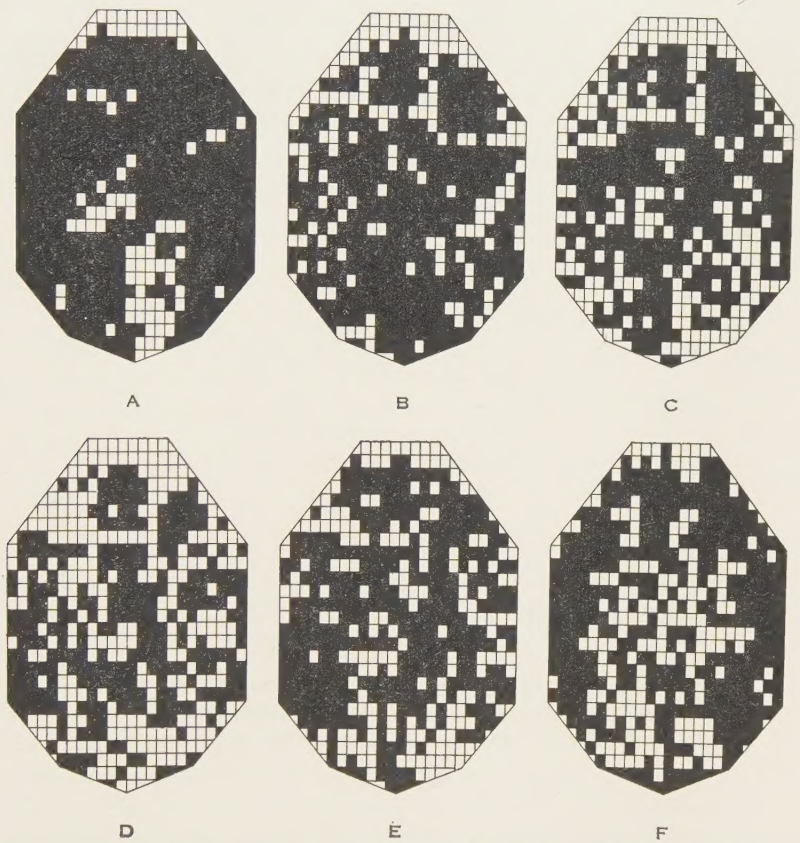

Fig. 5.—Distribution of *P. serrata* on the mouse after grooming has been prevented. The black areas are those in which one or more eggs or lice were found. *A*, eggs; *B*, females; *C*, males; *D*, stage III nymphs; *E*, stage II nymphs; *F*, stage I nymphs.

and on the third (G_3) to 500–550. The body weights of all mice remained within the limits of normal fluctuations (Fig. 6), and their demeanour appeared normal.

Elizabethan collars were fitted around the necks of five other mice and the louse populations were permitted to increase to between 1000 and 4000. The collars were then removed. After 24 hr two mice were killed and adult lice and stage III and stage II nymphs were found predominantly on the foreparts of both mice (Table 2). The distribution of the stage I nymphs, however, did not show

this trend. Thus, after only 24 hr grooming the distribution of the lice was apparently returning to normal. The remaining three mice were examined 4 days later when it was found that the number of lice had been reduced to less than 500.

Prevention of self-grooming with the mouth permitted the number of lice to increase rapidly and allowed them to populate the whole of the body. The number of lice was reduced rapidly when grooming was permitted to recommence.

(*b*) *Technique of Grooming*

The efficiency with which eggs were removed from the hair suggested that grooming was carried out by a combing action of the teeth rather than by licking and nibbling. The upper incisors of the mouse are fused together, whereas there is considerable movement between the two lower incisors. This fact, together with the upward sweep of the head of the mouse when grooming, suggested that the lower incisors were used.

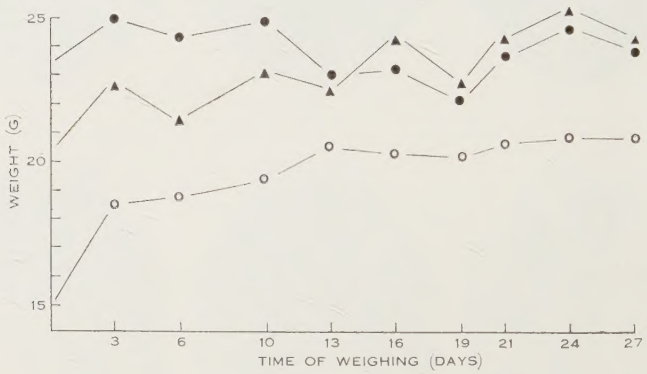

Fig. 6.—Body weights of mice during period when number of lice increased due to the prevention of self-grooming. ▲ Mouse G_1, 1000–1050 lice; ● mouse G_2, 1200–1250 lice; ○ mouse G_3, 500–550 lice.

A ring of polythene tubing was slid over one of the lower incisors of each of five mice and pushed down to the gum so as to wedge the teeth apart (Plate 1, Fig. 2). When the mice were examined three weeks later, the number of lice had increased and all stages were found on the hindpart of the body. It was apparent on visual examination that the number of lice was not as great as when Elizabethan collars were used to prevent self-grooming.

An Elizabethan collar was placed around the neck of a mouse and when the infestation had become heavy, the mouse was allowed to groom itself. One hour later it was killed, and many intact and mutilated lice and eggs were found in its stomach contents.

Lice, therefore, appeared to be removed by combing the hair between the lower incisors and were then ingested.

(c) *Effect of Reduction of the Efficiency of the Grooming Technique*

Ten mice were arranged in an ascending series according to body weight and were randomly divided into two groups. A polythene ring was placed around one of the lower incisors of each mouse in one group, after which they were all individually infested with 100–200 adult lice. The individual mice were kept in separate cages and examined regularly to ensure that the polythene ring was in position. All were weighed every 2 or 3 days until the experiment ended 38 days later, when the number of lice on each mouse was determined. During the course of the experiment, two mice in the experimental group died, and one in the control group. Figure 7 shows that the body weights of all mice remained fairly

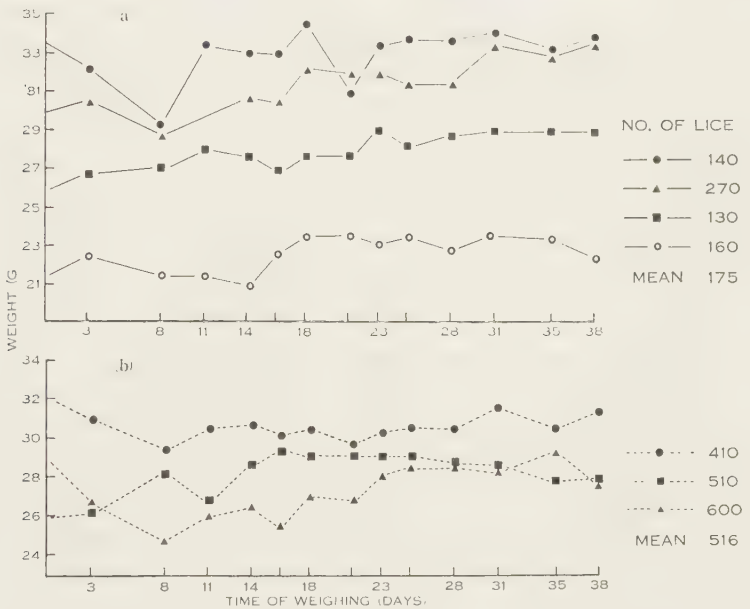

Fig. 7.—Body weights of mice during period when number of lice increased due to inefficient self-grooming. (*a*) Normal mice; (*b*) self-grooming rendered inefficient by placing a polythene ring around one of the lower incisors.

constant. There were, however, nearly three times as many lice on the experimental mice as on the controls, and numerous eggs and adult lice were found on the hindpart of the bodies of the experimental mice.

(d) *Effect of Reduction of the Area of the Body which can be Groomed*

Elizabethan collars were placed around the necks of three mice in such a manner as to prevent grooming of the left half of the hindpart of the body. Within 4 weeks the number of lice had increased from normal to over 1000, spread evenly over the region which could not be groomed, and only a few were found in the region which could be groomed.

Elizabethan collars were placed around the necks of another three mice to permit them to groom about three-quarters of the hindpart of the body. The number of lice increased but even after 42 days had not exceeded 500. Thus more than half of the hindpart of the body had to be groomed to control the number of lice.

(*e*) *Spread of* P. serrata *on Mice*

Elizabethan collars were placed around the necks of five louse-free mice and about 300 lice, predominantly adults and stage III nymphs, were placed on

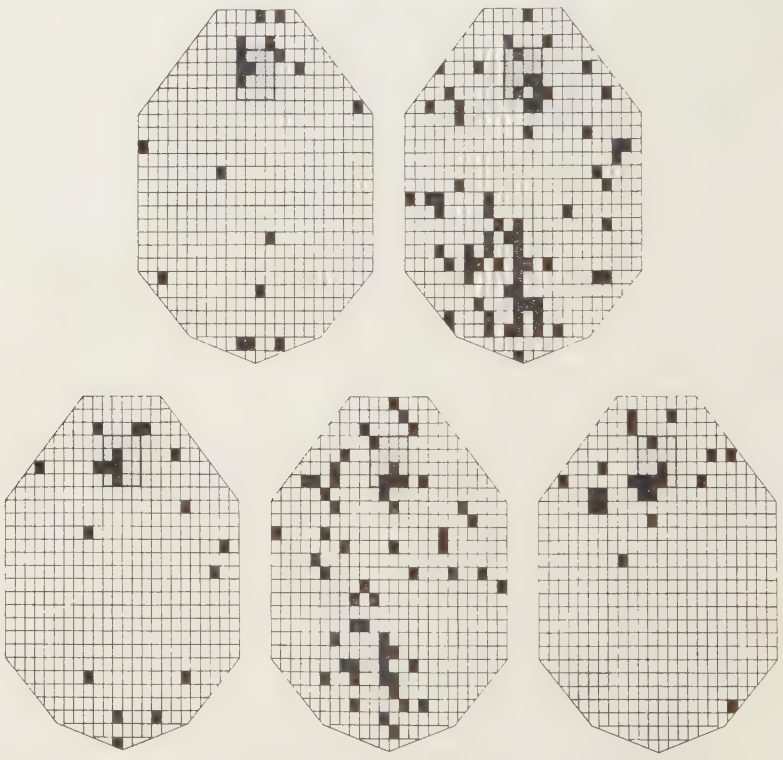

Fig. 8.—Distribution of *P. serrata* 24 hr after having been placed on the heads of mice. The stippled area, bounded by heavy lines, is the part of the head on which lice were placed. Self-grooming by the mice was prevented with Elizabethan collars. The black areas are those in which lice were found 24 hr later.

their heads. After 24 hr the mice were killed and lice were found over the whole body (Fig. 8), thus indicating that they wander readily and rapidly from the fore- to the hindpart of the body.

V. Discussion

Self-grooming was carried out primarily by combing the hair with the lower incisors and thus was limited to the part of the body which could be reached by

the mouth. This was principally the hindpart of the mouse and, as a result, the distribution of the lice was restricted. All stages of the life cycle were found predominantly on the forepart of the body with the exception of the stage I nymph which was found over the whole body. When Elizabethan collars were fitted around the necks of mice to prevent self-grooming, their louse populations increased from less than 200 to over 1000 in 3–4 weeks and all stages were found on the hindpart of the body.

Lice moved readily and rapidly from the forepart of the body to the hindpart, and thus it was possible for grooming of the hindpart of the body to control the number in the forepart. More than half of the hindpart of the body, however, had to be groomed to control the number of lice.

Both the prevention and the reduction of the efficiency of grooming allowed the lice to spread over the body. Therefore, the presence of any stage of *P. serrata* in numbers on the hindpart of the body is evidence of a decrease in the efficiency of its removal by self-grooming. Thus the preponderance of stage I nymphs over the other nymphal and adult stages and, in particular, their abundance on the hindpart of the body indicates that they were not being removed efficiently by combing with the teeth, probably due to their small size.

The basic requirements, therefore, for grooming to control the number of lice on mice and probably other rodents are:

(1) The technique should be efficient. This may vary with the size of each stage of the life cycle of the louse.

(2) The time spent grooming should be sufficient.

(3) The area of the body groomed should be adequate.

(4) The lice should not have predilection sites and should wander readily and rapidly into the region which is groomed.

If any one of these requirements is adversely influenced the number of lice will increase. Should the influence of such a factor be relatively slight, the number of lice will increase but will still be controlled and their distribution will become less restricted. The nature of the factors which influence these requirements may vary greatly, as may be seen in the following discussion of previous observations on louse populations on other rodents.

Experiments have been carried out on the influence of deficiencies of vitamins A (Searls and Snyder 1939; Kartman 1942) and B_6 (György 1938; Holmes 1958) in the diet of rats, and the increase in the number of lice on the rats on these deficient diets has been taken as indicative of a direct relationship between vitamin deficiency and the size of the louse population. In all these experiments, the rats became listless and showed definite symptoms of a vitamin deficiency. In this state, as Buxton (1947) has suggested, rats probably spend less time grooming which could account equally for the increase in the number of lice. In the present experiments, the body weights and demeanour of mice fed on an adequate diet remained normal whilst their louse populations increased rapidly when the efficiency of grooming was deliberately impaired (Figs. 6 and 7), thus demonstrating that grooming is the primary factor controlling the number of lice on mice.

The distribution of lice on some of the wild rodents of the Volga Delta has been described by Dubinin (1953). On *Apodemus agrarius* (Pall.), *Arvicola terrestris* (L.), and *Micromys minutus* (Pall.) the lice were restricted to the head and shoulder-blade region, and on *Mus musculus* and *Microtus arvalis* to the head and the root of the tail. These parts of the body cannot be reached by the mouth or only with difficulty.

Vysotskaya (1950) studied the fluctuations of *Hoplopleura acanthopus* on *M. arvalis* and found that the number of lice increased during the latter part of winter and declined in the summer. The cause of the decline was the loss of lice with the winter coat as it was shed. She found also that they were confined to the forepart of the body in the autumn, and in winter to around the root of the tail also, but in the spring they were found all over the body. She attributed this to behavioural responses of *M. arvalis* to the surrounding atmospheric temperature, such as sleeping in a curled-up position in winter. A decrease in the efficiency of grooming offers an alternative explanation for both the increase in numbers and the change in the distribution of the lice. This could be due to the increased thickness of the winter coat reducing the efficiency of the grooming technique, to less time being spent grooming because of the increase in the foraging and breeding activity, or to other factors associated with the conditions of stress to which the voles are exposed at this time. The findings of Cook and Beer (1958) with infestations of *H. acanthopus* on the meadow vole *M. pennsylvanicus* could be explained similarly.

As rodents become larger than mice the surface area of the body which has to be groomed increases. It might be anticipated, therefore, that grooming as carried out by mice may fail eventually to control the number of lice on larger rodents unless the grooming technique is improved. However, should there be differences in the rapidity with which the various species of lice can multiply on large or on small rodents, a less efficient grooming technique may be adequate when the rate of multiplication of the louse is less. On the rat, *R. norvegicus*, the louse *Polyplax spinulosa* Burm. is dispersed over the dorsal and lateral aspects of both the fore- and hindpart of the body (Dubinin 1953; Holmes 1958), indicating a decrease in the efficiency of grooming with the mouth which, however, may be due to factors other than the greater surface area of the rat. The average number of *P. spinulosa* on rats was found to be about 400 by Holmes (1958) and 500–600 by Zakovich (1946) which is only 4–5 times greater than the average number on mice. Thus the densities of the louse populations on rats and mice do not appear to differ greatly which suggests that the number of lice on the rats is being controlled efficiently. The life cycle of *P. spinulosa* requires 3–4 weeks to complete (Holmes 1958) and consequently it is unlikely that *P. spinulosa* can multiply as rapidly as *P. serrata* whose life cycle requires only 2 weeks for completion. Thus self-grooming is probably the main factor controlling the number of *P. spinulosa* on the rat also, even though the technique is apparently less efficient. It may well be that the greater efficiency of self-grooming by the mouse has caused selection for lice with a shorter life cycle.

ECOLOGY OF POLYPLAX SERRATA

Fig. 1.—Elizabethan collar of copper wire placed around the neck of a mouse.
Fig. 2.—Ring of stout polythene tube placed around the left lower incisor of a mouse. wedging the lower incisors apart.

Self-grooming is the principal factor which controls the distribution and abundance of *P. serrata* on the mouse *Mus musculus*, and probably also of lice on other small rodents. Many diverse factors can influence its efficiency which may be expected to decrease as the size of the rodent increases. It is necessary, therefore, in any study of the factors which cause fluctuations in the number of lice on rodents to take the effect of self-grooming into account.

VI. Acknowledgments

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AGE ESTIMATION BY MEASUREMENT OF POUCH YOUNG OF THE QUOKKA (*SETONIX BRACHYURUS*)

By J. W. Shield* and Patricia Woolley*

[*Manuscript received September* 27, 1960]

Summary

Removal of pouch young of *Setonix* can lead to the resumption of development of quiescent blastocysts. Thirty-six pouch young obtained in this way have been used in this study. As the time in days from resumption of development to parturition is known, accurate ages may be calculated without actual birth or copulation being observed.

These 36 animals have been repeatedly measured during their pouch life, and body weight, pes length, and tail length over the full term of pouch occupancy are given as regressions of these measures versus age. Larger-scale regressions are given for the first 80 days of pouch life. A tabulation based on the three regressions and giving average measures at stated ages is also given.

During pouch life there is no difference in growth rate between male and female pouch young. Growth proportions of field-reared and compound-reared animals of comparable nutritional status are also similar. It is therefore considered that growth rates are equal, and that the age-estimation procedure established on compound-reared animals is applicable to field animals.

I. Introduction

The first detailed study of the growth of marsupial young appears to be that of Hartman (1928) on the American opossum. During the past decade there have been several studies on the growth rate of marsupial young in the pouch (Reynolds 1952; Hill and Hill 1955; Dunnet 1956; Lyne and Verhagen 1957). None, however, are of macropod species. A variety of current studies in this Laboratory demand accurate age determinations both of quokka pouch young born in captivity, and those taken in the wild. This study presents age regressions for three easily measured characters of quokka (*Setonix brachyurus*) pouch young reared in compounds, and gives an assessment of the relative accuracy of each measure over the term of pouch occupancy.

Accurate aging of quokka pouch young reared in captivity may be accomplished by:

(1) Repeated examination of the pouch of previously mated females to determine the actual day of birth; or

(2) Discard of initial pouch young in order to stimulate quiescent blastocysts to resume development (Sharman 1955) and so produce second pouch young 25 or 26 days (Shield and Woolley 1960) after removal of the initial young.

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The first method entails the daily examination of all females in a breeding colony; concealment within the pouch immediately after birth necessitates daily handling, and results in pouch disturbance with consequent hazard to the newly born young. The use of pouch young developed from quiescent blastocysts avoids the daily examination of females near parturition whilst allowing birth to be predicted to within 1 day, so this method was used. The difficulties of aging pouch young reared in the field are considered in Section IV.

II. Methods

All the quokkas used in this study were taken from Rottnest I., W.A. (Hodgkin and Sheard 1959). Females with a pouch young were trapped, using

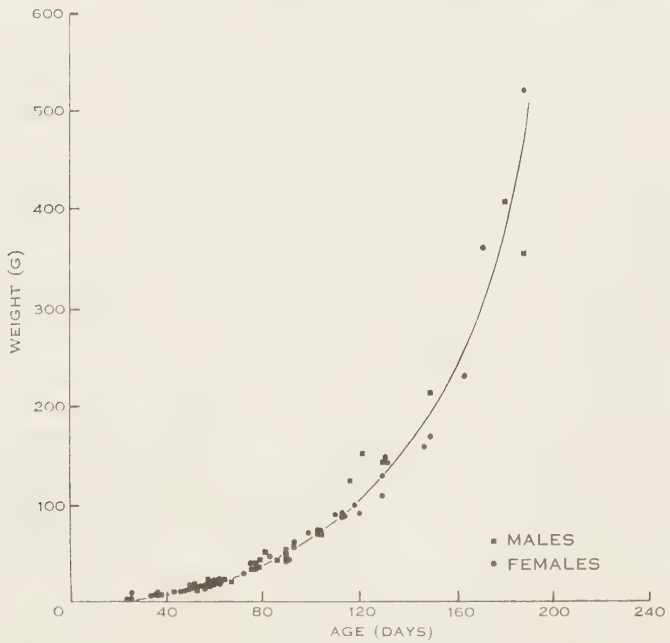

Fig. 1.—Regression of body weight versus age of pouch young of the quokka. Results of 77 observations shown.

hand nets, at night and retained in jute sacks until the following morning. The mothers were then tagged with numbered monel metal ear-tags and the pouch young removed. Various observations and measurements, including weight and pes length, which will form part of a separate study, were made on the pouch young before they were discarded. The females without their pouch young were then taken to the mainland, where they were retained in compounds in isolation from males to await the birth of a subsequent pouch young derived from the quiescent blastocyst (Sharman 1955). Females subsequently having a "delayed" pouch young were retained further in individual enclosures during the time the young occupied the pouch. The birth date of the delayed pouch young was in all cases reckoned as 25 days after the removal and discard of the initial pouch young.

The estimate of the actual age may therefore err in being 1 day shorter, at the most, than the actual age. These delayed pouch young then became the subjects for the regressions of age versus weight, pes length, and tail length.

Weighing and measuring of the pouch young was all done by one author (J.W.S.) whilst the other held the animals. Weights were taken using Salter spring balances which were periodically checked against standard laboratory weights. The pes length was in all cases measured on the right foot with a vernier caliper in the manner indicated by Wood Jones (1923), i.e. overall length of the plantar surface but excluding the nail. The tail was measured with a steel rule from the tip along the ventral surface to the junction with the body whilst the young animal

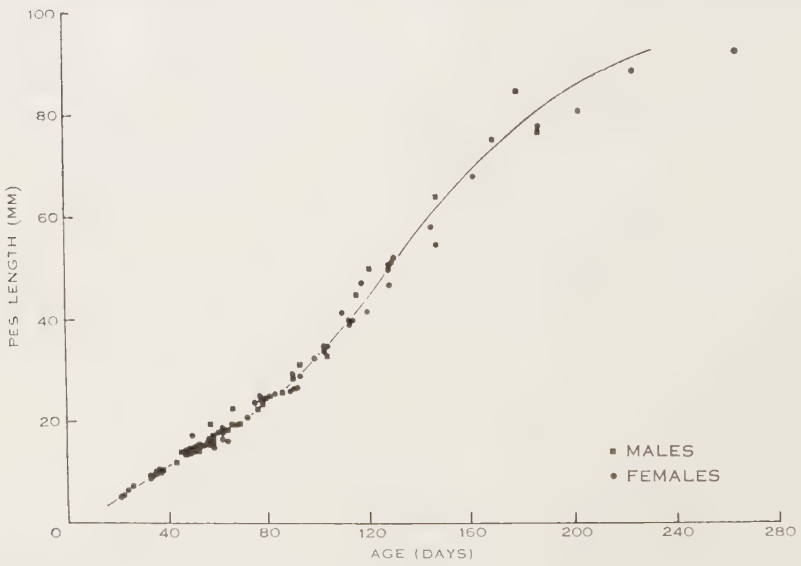

Fig. 2.—Regression of pes length versus age of pouch young (92 observations).

was suspended under its own weight by the tail. Weights under 100 g were measured to the nearest gram, and weights over 100 g to the nearest 5 g. The pes was measured to the nearest 0·1 mm, and the tail to the nearest millimetre.

Most animals were not examined for the presence of a delayed pouch young until the 28th day after removal of the initial pouch young in order to avoid disturbing the newly born young. Further, pouch young were handled with great caution; none were detached from the teat under 20 days of age, and when older animals were replaced in the pouch after measurement, the mother was kept tranquil in a sack for at least half an hour before release to ensure reattachment of the pouch young. After this procedure the young were nearly always retained in the pouch rather than being thrown out as often happened in the nervous reactions of the mother if she was released immediately. However, some wastage did occur immediately after handling.

III. Results

A total of 36 pouch young produced by the delayed-birth process were measured at intervals during their pouch life, and these measurements are presented as regressions of age versus weight, pes length, and tail length (Figs. 1–3).

The superimposed trend lines were fitted by eye, and drawn with the aid of French curves. Males and females have been presented separately in the regressions to show that during pouch life there is no sex difference in these

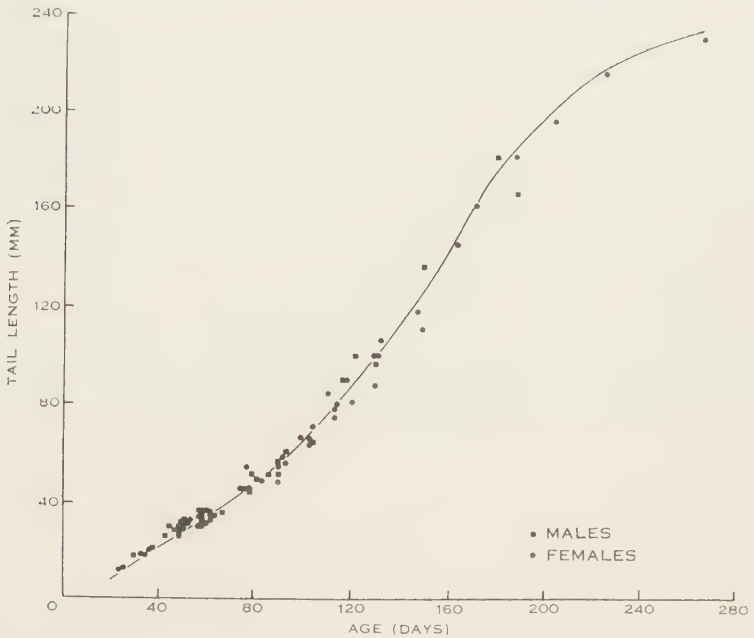

Fig. 3.—Regression of tail length versus age of pouch young (80 observations).

measures. Table 1, which gives the average weight, pes length, and tail length for a given age pouch young, has been compiled from these regressions. Figures 4–6 are enlargements of the first 80 days shown in Figures 1–3.

Figures 7 and 8 are regressions of cube root of the weight versus pes length for the initial pouch young (field-reared), and the delayed pouch young (compound-reared) respectively. Figure 7 contains the measurements of about 300 pouch young measured once only, while Figure 8 contains the measurements of 36 pouch young, some of which have been measured several times. The regression line of Figure 8 is drawn identical with that of Figure 7, and has not been separately fitted to the Figure 8 data. This regression line of Figure 8 may be used to convert pes length to weight or vice versa.

IV. Discussion

Whether field populations or domesticated colonies of the quokka are used, several difficulties attend the establishment of a method for accurate age estimation

Table 1

AVERAGE WEIGHT, PES LENGTH, AND TAIL LENGTH OF QUOKKA POUCH YOUNG OF GIVEN AGE

Age (days)	Weight (g)	Pes Length (mm)	Tail Length (mm)	Age (days)	Weight (g)	Pes Length (mm)	Tail Length (mm)
10	1·5	—	—	70	28·5	20·5	42·0
15	2·0	3·5	5·0	80	38·3	24·0	48·5
20	2·5	5·0	8·5	90	52·0	28·0	56·5
25	3·0	6·5	12·0	100	67·0	32·0	65·5
30	4·7	8·0	15·5	120	106·0	43·0	88·0
35	5·5	9·5	19·0	140	167·0	55·5	115·0
40	8·3	11·0	22·0	160	—	67·0	145·0
45	9·7	12·5	25·0	180	—	77·0	175·0
50	14·3	14·0	28·5	200	—	84·0	199·0
60	21·0	17·0	35·0				

of pouch young. In both cases, a hazard to the young exists in handling and detachment from the teat for measurement, and unless extreme caution is taken a

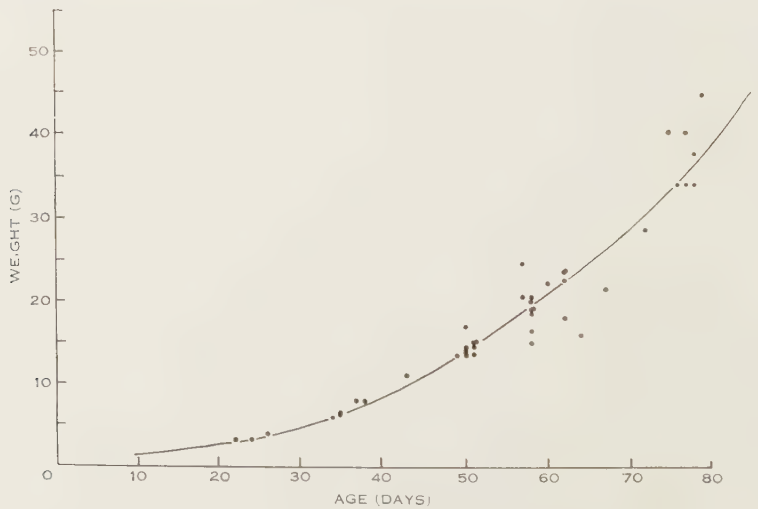

Fig. 4.—Regression of age up to 80 days versus body weight of pouch young.

proportion of such examinations result in premature loss of the pouch young. Also, in view of the individual variability of the various measures of age, the

sample studied must be sufficiently large to establish regressions representative of the population. Further, the equivalence of age estimations cannot be assumed.

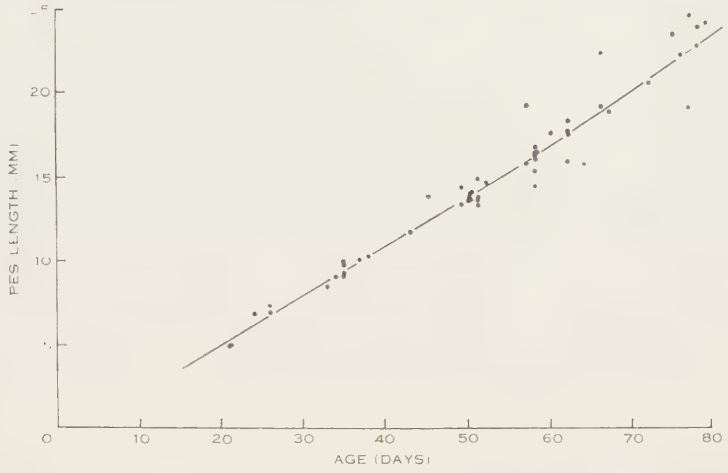

Fig. 5.—Regression of age up to 80 days versus pes length of pouch young.

Before age-estimation procedures established on compound-reared animals can be strictly applied to field populations, it must be shown that growth proportions as well as growth rates in the field and in captivity are indeed comparable.

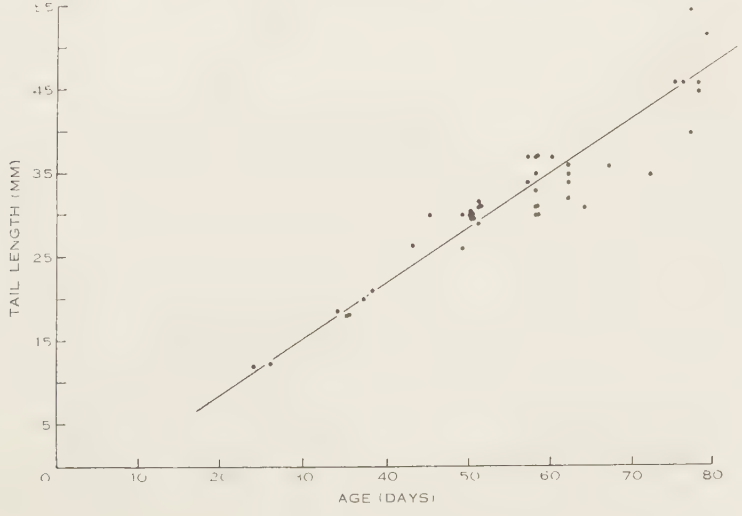

Fig. 6.—Regression of age up to 80 days versus tail length of pouch young.

Some estimate of the age of field-reared pouch young can be obtained by the method of marking with subsequent repeated recapture. If a small pouch

young appears in the pouch between two recaptures, then the age of the pouch young cannot be greater than the time between recaptures of the mother. By measuring a number of individual pouch young at subsequent recaptures an estimate of age with a measurable error can be obtained. The accuracy of this method is, however, primarily determined by the time interval between the first two recaptures. An alternative method based on the assumption that pouch young of near minimum size are close to their birth dates is inaccurate as weight increase in the first 20 days of pouch life is very slow. In practice neither of these two methods can fully resolve the actual birth date. Also, in both cases, the accuracy is directly dependent upon the numbers caught and the frequency of

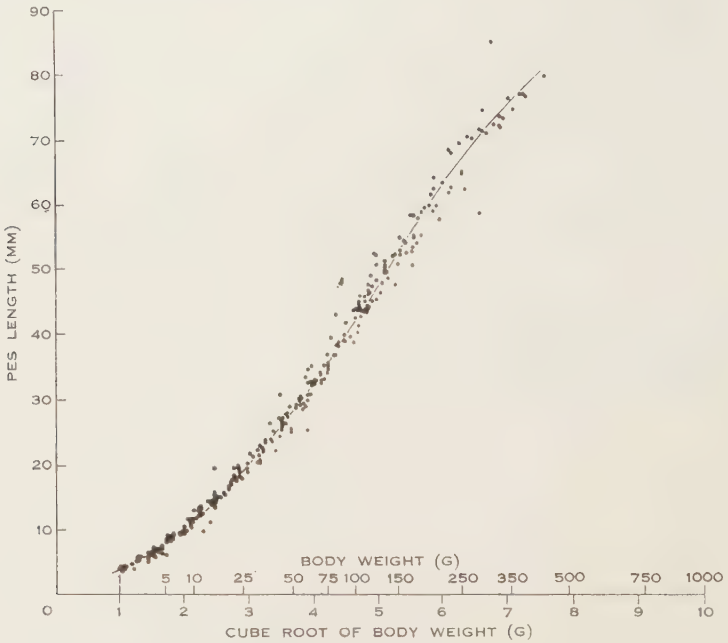

Fig. 7.—Regression of pes length versus cube root of body weight of pouch young reared in the field.

recapture. With low recapture percentages generally encountered in large mobile populations these methods become impracticable. A further error may occur if the early growth stages are not observed at intervals less than the gestation time for delayed birth (25 days). Replacement of the original pouch young could occur between recaptures, giving the appearance of continuous growth of a single pouch young. It is also known from observations on compound-reared animals, that females losing a large pouch young endeavour to acquire another one from other females, and have on occasion been successful in this manoeuvre. Hartman (1928) also mentions that errant young have been known to find foster mothers in *Didelphis*. These substitution errors can easily be detected in compounds but not in the field. The practical limitations of age estimation in the field are thought to

be restrictive, hence the procedure of obtaining pouch young of known age by the delayed-birth process in the compounds.

Mathematically fitted curves relating growth to actual age are sometimes thought to be desirable from several points of view. They are said to be consistently accurate, taking into account each and every observation, and as such best serve as a basis for comparison with similar data. However, in general, growth rates follow no simple and obvious mathematical curve, and the gratuitous assumption of one type of curve to which the data is fitted by the method of least squares rather than another type can have no justification other than computational convenience, and may be definitely misleading. Simpson (in Jepson, Mayr, and Simpson 1949) questions whether the fitting of a mathematical curve is justified,

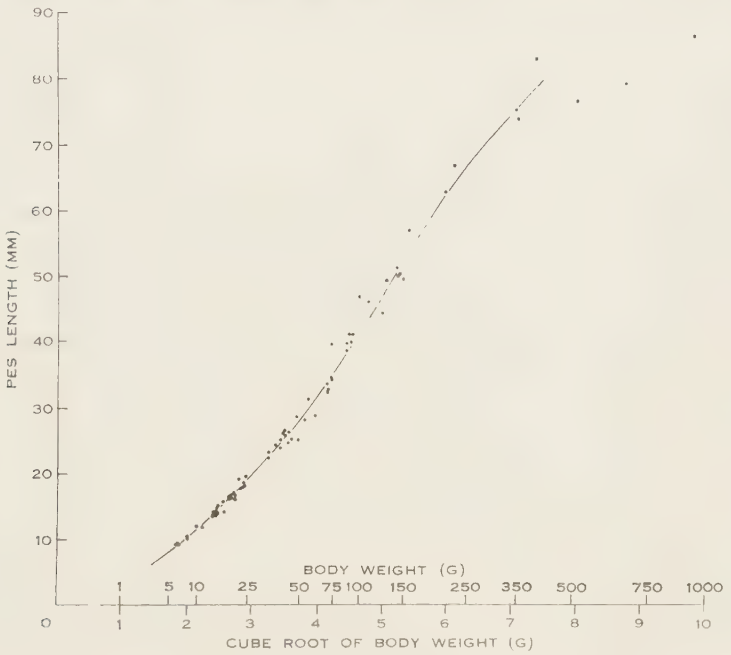

Fig. 8.—Regression of pes length versus cube root of body weight of pouch young reared in captivity.

regardless of the labour involved, unless the constants can be assigned some biological meaning. Waddington (in Zuckerman *et al.* 1950) also thinks that the growth formulae derived in this manner have very restricted application. Extrapolation beyond the data range is risky. No causal relations can be deduced from the formula, and differing biological systems can only be compared in a strict mathematical sense. Finally, the observations contained in the present regressions are repeated measurements of young throughout their pouch life, and hence each observation is not statistically independent. Simply fitted curvilinear regressions assume such independence. Accordingly, all the regressions have been fitted by eye, and drawn with the aid of French curves, and are not intended as mathematical comparisons with other growth curves.

Upon inspection of Figures 1-3 it is apparent that the regressions are not equally efficient for age estimation over the whole length of pouch life (approximately 180 days). The accuracy of each regression for estimating age depends upon both the slope of the trend line, and the variability about the line. For the first 80 days the weight rises very slowly, while the pes and tail lengths rise sharply. Since the variability is about the same, the pes and tail length regressions give greater precision in the assessment of age during this period. In the intermediate range of 80–120 days the three measures have comparable slopes and variability, and appear to be equally reliable for age estimation. After 120 days the variability of all three measures increases, and consequently the general reliability decreases, Weight rises abruptly after 140 days and with the inherent variability becomes a poor criterion of age. Pes length and tail length increase less rapidly than weight, and are the best measures for estimating the age of young older than 120 days. It is suggested that, in assessing the age of any particular pouch young, the ages corresponding to the three measures be averaged for greater accuracy. The age corresponding to each measure can be obtained from either the regressions, or from Table 1, which is sufficiently detailed for linear interpolation. Comparison of the three growth curves of the quokka pouch young with other marsupial young data shows certain similarities. Weight increase with age follows a similar trend in *Didelphis* (Hartman 1928), *Trichosurus* (Lyne and Verhagen 1957), and *Setonix*. For *Setonix* and *Trichosurus* which accommodate only one young in the pouch the abrupt rise in body weight corresponds to the time when the young is ready to leave the pouch. Pes and tail growth rate curves also are similar in *Trichosurus* and *Setonix*. Both curves are somewhat sigmoidal but maintain an average rising slope with no sharp increases over the period of pouch life.

Figures 7 and 8 are regressions of the cube root of weight versus pes length for field- and compound-reared pouch young respectively. Figure 8 shows that the regression points of the compound-reared animals are well represented by the superimposed Figure 7 regression line, and as there is no difference in slope, shape, or variability they are tendered as evidence that the growth proportions of field- and compound-reared pouch young do not materially differ. It is considered most probable that the growth rates of pouch young in the field and in the compounds are similar since field pouch young develop during the winter and early spring, when the vegetation is relatively lush and conditions are best. Compound-reared animals likewise have an adequate diet, with food and water always in excess of requirements. Although equality of growth proportions can be fairly simply verified, little can be done without extensive mark and recapture work to statistically validate equal growth rates between field- and compound-reared pouch young. However, with equal growth proportions developed in field- and compound-reared animals both on seemingly adequate diets, it appears most probable that growth rates are likewise similar.

V. Acknowledgments

The authors wish to thank several members of the Zoology and Physiology Departments of the University of Western Australia who helped to catch the

animals on Rottnest Island. Mr. A. J. Fraser, Chief Warden, Fisheries and Game Department, Perth, gave the necessary permits to take the animals. The animal husbandry of Mr. C. Chekanouskis has greatly contributed to the success of the work. Professor H. Waring has materially helped as well as giving critical comment during the tenure of the study.

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MORBIDITY AND MORTALITY IN THE KOALA (*PHASCOLARCTOS CINEREUS*)

By T. C. Backhouse* and A. Bolliger†

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Summary

In all, 28 koalas (*Phascolarctos cinereus*), 26 of which had died exclusively of natural causes, were autopsied and in 21 of these a probable cause of death could be recognized.

Different forms of pneumonia head the list of these causes with six cases, including two where the primary lesion was trauma. Hepatitis with suppurative cholangitis was observed in three instances. Cryptococcosis, an infection by the yeast *Cryptococcus neoformans,* was responsible for three deaths, and two forms of blood dyscrasia, i.e. lymphoblastic leukaemia, and an anaemia of unknown origin accounted for two more deaths. Cystic disease of the ovary was observed in six koalas, and in four cases was complicated by infection and was the main cause of death. Middle ear sepsis, ulcerative colitis, and cardiac failure associated with senility were seen once each.

In the remaining seven cases the cause of death was indeterminate, though senility appeared to be the predisposing cause in two.

I. Introduction

During the past forty years a number of papers have been written describing various aspects of koala life — habits in captivity, diet, distribution in different regions, threatened extinction, and methods of conservation, etc. A few papers have described the results of anatomical studies (Mackenzie 1919; Pocock 1921; Sonntag 1921), still fewer have dealt with some aspects of its physiology (Bolliger 1953), and more recently some blood studies have been carried out (Bolliger and Backhouse 1960).

With the exception of an interesting account of an outbreak of respiratory infection in koalas in Queensland (Rahman 1957), the present authors have been able to find only passing mention of pathological conditions in this animal, and so have thought it worth while to report those encountered while the blood studies were proceeding and subsequent to that period. The living and dead koalas available for these studies were all at Taronga Park Zoological Gardens, Sydney, although many were recent acquisitions and some had died within a day or so of reception from their native haunts. However, no attempt has been made to compare the relative merits of captivity and life in the bush as affecting longevity.

II. Pathological Findings

Post-mortem examinations on the bodies of some 30 koalas supplemented by the histological study of many microscopic sections of tissues have revealed

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a variety of pathological lesions, and, although in some instances the determining cause of death remained obscure, in more than two-thirds it could be stated with a fair degree of confidence while the remainder had to be relegated to the category of "ill-defined diseases".

This could hardly have been otherwise in the absence, in most cases, of ante-mortem clinical and laboratory studies, particularly bacteriology. An added cause of confusion was the occurrence of putrefactive and autolytic changes. Omitting those animals in which such changes were too advanced for any useful histological studies, we were left with 28 in which were found the pathological conditions and the causes of death as set out in Table 1. Some of these conditions call for further comment under the following appropriate headings.

(*a*) *Pneumonia*

It is not known whether any of the six cases included in this heading were of pneumococcal origin. None showed frank lobar consolidation although two (K4 and K23) may have gone on to further consolidation by confluence had the animals survived for longer periods. Of the remaining four, one, K48, showed a compressed cornified lung with empyema as a complication. Regarding the Gram-positive bacilliform organisms noted in Table 1 these resembled diphtheroids in appearance, a point of some interest in view of Rahman's (1957) findings in Queensland where *"Corynebacterium koala"* appeared to be causally related to the respiratory infection and was isolated from the lungs of the animal which died.

The remaining three showed active hyperaemia with death at an early stage. K56 showed this condition in an extreme degree with effusion of blood into the interstitial tissues between the alveoli.

(*b*) *Hepatitis*

Three koalas (K1, K17, and K51) showed definite suppurative inflammation and necrosis in the liver, K51 having, in addition, enlarged suppurating mesenteric glands while K17 had presented marked anaemia ante-mortem as already mentioned in another paper (Bolliger and Backhouse 1960).

In liver sections, generally, it has been noted that the cells are remarkable for the amount of brownish yellow pigment, presumably bile. Dr. H. A. Ball (personal communication), who autopsied a koala at San Diego Zoological Hospital, also commented on the remarkable deposition of bile pigment in the liver.

(*c*) *Blood Dyscrasias*

Two koalas showed evidence, however incomplete, of blood disorders which seemed deserving of further mention in view of the rarity with which they have been recorded in wild animals (Engelbreth-Holm 1942). K2 was a female koala seen at autopsy only. The spleen was enlarged (weight 30 g, normal range 4–8 g) and there was *c.* 200 ml of free fluid in the peritoneal cavity. Films from the heart blood stained with Leishman stain showed a striking picture dominated by large numbers, in every oil-immersion field, of cells of the lymphocyte series. Many

TABLE 1

AUTOPSY AND HISTOLOGICAL FINDINGS

No.	Sex	Autopsy Notes	Principal Histological Findings in Brief	Probable Cause of Death
K1	♂	Ascites; pleural effusion	*Liver.*—Engorgement of sinusoids, patchy necrosis of liver cells, small haemorrhagic foci infiltrated with leucocytes, many bile canaliculi packed with polymorphs. Some portal cirrhosis, no regeneration of liver cells	Hepatitis, suppurative cholangitis
K2	♀	Ascites; spleen enlarged	See text	Lymphoblastic leukaemia
K4	♂	Patchy red consolidation of lungs	*Lung.*—Many alveoli filled with red blood cells and polymorph leucocytes	Pneumonia
K7	♂	Ascites; pleural and pericardial effusions; spleen and mesenteric lymph nodes enlarged	See text	Anaemia of unknown origin
K17	♀	Ascites; *c.* 250 ml free fluid in peritoneal cavity	*Liver.*—Similar to K1 above, viz. necrosis of liver cells and collections of polymorphs in bile ducts. Early portal cirrhosis. Blood haemoglobin prior to death 6·2 g/100 ml	Hepatitis, suppurative cholangitis; anaemia
K23	♂	Free fluid in pleural cavity	*Lung.* Extreme engorgement of alveolar capillaries, scattered patches of "red hepatization" *Liver.*—Engorged sinusoids. Irregular thickening of capsule. Some increase in fibrous tissue of portal canals *Spleen.*—Great thickening of trabeculae, distortion of lymphoid follicles. Blood prior to death contained 14,000 leucocytes per cu. mm and 60% neutrophils	Pneumonia
K28	♂	Multiple lacerations of skin; dislocation of shoulder; marked congestion of lungs	*Lung.*—Intense engorgement of alveolar capillaries obscuring cellular structure *Liver.*—Engorged sinusoids. Yellow granules in liver cells and grey-green staining granules in Kupffer cells *Spleen.* Fibrous trabeculae grossly thickened, very little lymphoid tissue remains *Blood* (prior to death).—See Bolliger and Backhouse (1960)	Trauma; infection; early pneumonia

TABLE 1 (*Continued*)

No.	Sex	Autopsy Notes	Principal Histological Findings in Brief	Probable Cause of Death
K41	♂	An old koala, no obvious lesions	*Liver.*—Some capsular thickening and increased collagen in portal canals. Much yellow-brown pigment in liver cells *Spleen.*—Fibrous trabeculae prominent. Sinuses engorged, lymph follicles few	No obvious cause, probably senility
K42	♂	An old koala, no obvious lesions	*Liver.*—Sinusoids congested. Cells with yellow pigment as in K41 *Blood* (prior to death).- See Bolliger and Backhouse (1960)	Senility; cardiac failure
K43	♀	Pancreas appeared engorged, with some haemorrhages	*Liver.*—Sinusoids engorged. Kupffer cells contain grey-green granules *Kidneys.*—General engorgement, small haemorrhages, tubular epithelium desquamated *Adrenals.*—Medullary cells widely separated by haemorrhage *Spleen.*—Fibrous trabeculae prominent. Lymphoid follicles irregular but fairly numerous. Many deposits of brownish pigment and histiocytes contain similar granules *Pancreas.*—General engorgement of vessels and some small areas of haemorrhage. No inflammatory changes	Obscure. General haemorrhagic tendency, e.g. into adrenals, pancreas, etc.
K46	♀	Broken leg; multiple lacerations of skin; infected wounds	*Sections.*—None examined *Blood* (prior to death).—See Bolliger and Backhouse (1960)	Trauma; sepsis
K48	♂	Right lung collapsed and compressed by large collection of purulent fluid; lung adherent to diaphragm	*Lung.*—Section where adherent to diaphragm shows marked fibrous thickening of pleura which is infiltrated with polymorphs and red cells. The lung tissue is compressed and cornified. Many masses of Gram-positive bacilli surrounded by zones of leucocytic infiltration	Pneumonia; empyema
K49	♂	General congestion of organs, no special lesion noted	Liver, kidney, adrenals, and pancreas all showed capillary engorgement and tissues contained proliferating masses of mixed bacteria	Obscure. Bacterial invasion probably terminal or post-mortem occurrence

TABLE 1 (*Continued*)

No.	Sex	Autopsy Notes	Principal Histological Findings in Brief	Probable Cause of Death
K50	♀	Enlarged spleen, no other gross changes noted	*Liver.*—Sinusoids engorged, Kupffer cells prominent, many showing ingested cellular debris. Much yellow pigment in liver cells *Kidney.*—Intense engorgement *Adrenals.*—Cells in all zones widely separated (? oedema). Some increase of collagenous hyaline material in zona glomerulosa. Medulla poorly defined *Spleen.*—Capsule thickened. Fibrous trabeculae increased. Few sparse lymphoid follicles. Microscopic field is largely occupied by erythrocytes with relatively few mononuclear elements	Obscure
K51	♀	Large masses of mesenteric and retroperitoneal lymph glands softened and suppurating; *c.* 1200 ml free fluid in peritoneal cavity	*Liver.*—Portal canals infiltrated with neutrophils and lymphocytes. Liver cells contain angular granules of brown pigment negative for prussian blue reaction. Kupffer cells contain granules grey-green in haemotoxylin and eosin section and giving positive prussian blue reaction *Kidney.*—Widespread destruction of epithelium of convoluted and collecting tubules *Adrenals.*—Cortical zones relatively normal. Medulla appears somewhat fibrotic *Spleen.*—Marked increase in fibrous trabeculae, follicular structure lost, many phagocytes containing brown pigment *Mesenteric lymph nodes.*—Follicular structure obliterated by neutrophil leucocytic infiltration	Hepatitis; suppurative cholangitis; mesenteric adenitis

TABLE 1 (*Continued*)

No.	Sex	Autopsy Notes	Principal Histological Findings in Brief	Probable Cause of Death
K52	♂	Excess fluid in peritoneal cavity (50-100 ml); enlarged mesenteric lymph nodes; some haemorrhages on pericardium and in parietal pleura along ribs; haemorrhagic consolidation at base of right lung	*Lung.*—Intense general engorgement of alveolar capillaries. No actual consolidation seen in section examined *Liver.*—Cells vacuolated and contain yellow granules. Large clumps of bacilli present- some within swollen Kupffer cells *Kidney.*—Diffuse autolysis of renal epithelium, nuclei stain poorly *Adrenals.*—One gland showed extensive haemorrhagic areas *Spleen.*—Fibrous trabeculae and red cells occupy most of section. Very few lymphoid follicles	Early pneumonia
K53	♀	Large unilateral ovarian cyst containing purulent fluid	*Ovary.*—Serous cystadenoma; wall infiltrated with leucocytes	Infected ovarian cyst
K54	♀	Numerous ulcers in colon; some depressed scars on kidneys	*Liver.*—Thickening and hyalization of capsule. Increased collagen in portal canals and arteriolar walls. Scattered foci of lymphocytes and plasma cells *Kidneys.*—Widespread destruction of tubular epithelium. Some irregular crowding of glomeruli but no obvious interstitial fibrosis *Adrenals.*—Medulla shows some hyaline collagenous tissue *Pancreas.*—Engorgement and some increase of interstitial fibrous tissue	Ulcerative colitis
K55	♀	Large multilocular cysts in both ovaries, one ruptured; intestinal adhesions	*Ovary.*—Typical pseudomucinous cystadenoma	Large size of cysts and partial intestinal obstruction due to adhesions
K56	♂	An old koala, lungs appear haemorrhagic	*Lung.*—Extreme congestion of capillaries. The whole section appears to consist of erythrocytes, nuclei of tissue cells being inconspicuous *Liver.*—Intense congestion of sinusoids *Kidneys.*—Congestion and haemorrhages	Haemorrhagic pneumonia

TABLE 1 (*Continued*)

No.	Sex	Autopsy Notes	Principal Histological Findings in Brief	Probable Cause of Death
K57	♀	Lungs pale, oedematous; numerous white nodules present	*Lung.*—Nodules show capsulated fungal organisms morphologically resembling *Cryptococcus neoformans* (see text)	Pulmonary cryptococcosis
K58	♀	Brain bulging, oedematous and hyperaemic; ovarian cyst	*Leptomeninges.*—Infiltrated and show cryptococci as in K57 (see text)	Cryptococcal meningitis and encephalitis
K59	♀	Small white nodule near base of one lung; large mediastinal mass adherent to roots of great vessels and trachea	*Lung.*—Sections of lung nodule and mediastinal mass show numerous organisms as in K57 and K58 (see text)	Pulmonary and mediastinal cryptococcosis; pressure effects of heart and great vessels
K60	♂	Middle ear cavities filled with pus; brain oedematous	No histological findings	Middle ear sepsis; toxaemia
K61	♀	Cystic ovaries and tubes	*Wall of cyst.*—Serous type of cystadenoma	Obscure
K62	♀	Cystic enlargement of ovaries; cysts contain pus as do also uteri and vaginal complex	*Wall of cyst.*—Inflammatory changes—destruction of epithelium. Apparently an infected serous cystadenoma (see text)	Infected cystadenoma of ovary; toxaemia
K64	♀	Bilateral ovarian cysts; right ovarian cyst infected and perforated	See text	Infected cystadenoma of ovary

of these were immature, having deep blue cytoplasm, round or ovoid nucleus with coarse chromatin, and a conspicuous central or slightly eccentric single nucleolus. These cells closely resembled prolymphocytes. Smaller cells more like lymphoblasts and lymphocytes were also present. Similar cells were present in infiltrations in the portal canals of the liver and in the kidneys. The spleen section showed a loss of the normal follicular structure and every field was largely occupied by cells of the above-described types. One had, on the evidence, to conclude that this was a case of lymphogenous leukaemia showing a predominance of immature cells.

Koala K7, a fully grown but young male, was found in the bush, where it made no opposition to being captured. After its arrival at the Zoological Gardens, its blood picture was examined and it appeared that this koala was suffering from anaemia, its haemoglobin content being only half of the normal haemoglobin content established on a large number of native bears (Bolliger and Backhouse 1960). It also had an excessive number of normoblasts and reticulocytes. Furthermore, anisocytosis, poikilocytosis, central pallor, and Howell-Joly bodies were noted. Subsequently, 29 blood counts, most of them complete, were performed over a period of 7 months till the animal died. A number of these counts are listed in Table 2.

During this period, attempts were made to correct the anaemia by the administration of iron, vitamin B_{12}, folic acid, and liver extract. On the whole, this was unsuccessful, and after maintaining the original status for about 6 months, the health of the koala deteriorated in spite of any of the above-mentioned treatments. The erythrocyte content, prior to death, was only 500,000 per cu. mm.

On post-morterm examination, the abdominal cavity contained over 200 ml of greenish-coloured fluid. About 150 ml of fluid were in the chest cavity, and the pericardial sac was distended with blood-tinged exudate. The surface of the heart was covered with fibrinous exudate, which, in places, adhered to the pericardial sac.

Microscopic sections of the liver showed some central lobular necrosis, but no other significant changes. The spleen section showed great engorgement of the pulp sinuses, the lymphoid follicles being atrophic and sparse. The bone marrow smear, as with most such smears made after death, stained poorly and was difficult to interpret. Many normoblasts were present as well as numerous other immature cells of the "blast" type, some of which were considered to be lymphoblasts. Cells of the granulocyte series were very difficult to find.

The definition of the type of the anaemia in human terms was difficult but the blood picture suggested a haemolytic process.

(*d*) *Cryptococcosis**

It is noteworthy that three out of a total of 28 koalas autopsied showed well-advanced lesions of this fungus infection (K57, K58, and K59). Although of cosmopolitan distribution and reported as occurring in a variety of animals in other countries, it seems hitherto to have been limited to the human race in

* Synonyms torulosis, European blastomycosis.

TABLE 2

BLOOD COUNTS OF KOALA K7

Date	Haemoglobin (g/100 ml)	Erythrocytes per cu. mm ($\times 10^6$)	Nucleated Erythrocytes (per 100 leucocytes)	Reticulocytes (per 100 erythrocytes)	Leucocytes per cu. mm ($\times 10^3$)	Neutrophils (%)	Eosinophils (%)	Basophils (%)	Lymphocytes (%)	Monocytes (%)	Medication
1. xi.56	6·5	2·3	20		4·9	1	0	0	99	0	Iron
10.xii.56	6·2	2·1	78	6	9·8	2	0	0	97	1	Vitamin B_{12}
24.xii.56	5·1	1·9	27	7	8·3	5	1	0	92	2	Folic acid
23. i.57	7·8		58	5	9·0	6	1	0	91	2	Folic acid
5. iii.57	8·4	2·8	22	1	5·2	3	0	0	95	2	"Neo-Hepatex"
17. iv.57	6·9	2·3	25	1·4	4·4	2	0	0	97	1	
22. v.57	4·1	1·4	10	0·5	6·1	6	0	0	91	3	Vitamin B_{12}
5. vi.57	3·0	0·8	7	1·8	2·5	1	0	0	97	2	
12. vi.57		0·5		0·2	1·6						Dies

Australia (Cox and Tolhurst 1946). No cases in domestic animals were recorded by Seddon (1953) in his comprehensive service publication of that year, and so far as the authors are aware, it has not been reported in the native fauna.

The organism *Cryptococcus neoformans*, an asporogenous yeast, is widely distributed in nature, and in the United States of America has been isolated from pigeon droppings and also from the nests of these birds (Emmons 1955; Littman and Zimmerman 1956). This suggests transmission by dust via the respiratory route; direct transmission from animal to animal has not been demonstrated.

The three infections in koalas seen by the authors have already been the subject of a brief preliminary report (Backhouse and Bolliger 1960), but it is hoped that further mention of them here may stimulate interest and emphasize this added threat to the health of the koala and possibly other native fauna.

(i) *Koala K*57.—This animal, a young female, died at Taronga Park Zoological Gardens in April 1959. A post-mortem examination revealed pale oedematous lungs in which were numerous firm white nodules, sometimes forming confluent areas at the periphery of the lobes. No gross lesions were seen elsewhere in the body. Microscopic examination of sections of these nodules showed the lung alveoli packed with spherical organisms of variable size, from 5–10 μ in diameter, surrounded by a halo usually unstained in haematoxylin and eosin sections, but showing as a stained capsule with faint radial striations when stained with periodic acid–Schiff (P.A.S.) or examined under phase-contrast illumination (Plate 1, Fig. 1). For the most part, the alveolar septa were intact, but in parts broken down, producing irregular spaces filled with capsular material and organisms. In the specimens examined there was little evidence of the granulomatous infiltration often seen in human lungs, but there were scattered small foci of lymphocytes and histiocytes in the angular areas between groups of alveoli. Giant cells were few and there was very little fibroblastic proliferation. These appearances were consistent with those of *C. neoformans* infection. Plate 1, Figures 1 and 2, show typical areas in stained sections.

(ii) *Koala K*58.—In February 1960, a middle-aged, fully grown female was seen to have marked rotatory nystagmus and suffered from clonic convulsions, dying shortly afterwards. At autopsy the brain was found to be bulging, hyperaemic, and oedematous. Sections of various portions of the cerebrum, cerebellum, and spinal cord showed marked oedema and round-celled infiltration of the leptomeninges and many capsulated organisms lying in the tissue spaces. These had the dimensions and appearances of *C. neoformans*. Apart from the meningeal lesions, there were scattered granulomatous foci in the brain substance surrounding groups of cryptococci (Plate 1, Fig. 3). There were also sparsely scattered cryptoccocci in the substance of the cord rendered conspicuous by P.A.S. staining. In man, cryptococcal meningitis frequently follows lung invasion, but no obvious lung lesions or foci elsewhere were seen in this animal.

(iii) *Koala K*59.—This part-grown female was found dying in the bush so that infection is not necessarily contracted in captivity. One lung presented a single small firm white nodule near the diaphragmatic surface of the base (Plate 2, Fig. 1) and a mass (1·5 by 3 cm) of confluent nodules involving mediastinal glands

and adherent to the roots of the great vessels of the heart and to the trachea. Microscopic sections from the lung nodule and from the mediastinal mass showed histological features essentially similar to the foregoing cases. It is unfortunate that no culture studies were attempted in any of these three instances of cryptococcosis, so that the identity of the organism as *C. neoformans* rests on its appearances in tissue sections only.

III. Diseases of the Female Genital Tract

The anatomy of the female internal reproductive organs of the koala is typical of that of marsupials in general and particularly of that of Phalangeroideae. It consists of two ovaries, two uterine tubes, two entirely separate uteri, a median and two lateral vaginae (vaginal complex), and an elongated urogenital sinus. The necks of the uteri are inserted into the median vagina, which is also referred to as the cul-de-sac, owing to the lack of a caudal outlet. At parturition an opening at the distal end of the cul-de-sac appears and a temporary passage for the foetus is created (pseudovagina or birth canal) which leads into the urogenital sinus (Mackenzie 1919).

Lateral to the points where the uterine cervices enter the median vagina, the lateral vaginae communicate with the cul-de-sac. These are two tubes of comparatively narrow lumen, one on either side of the median vagina, extending and connecting with the proximal end of the urogenital sinus.

This short description demonstrates the complicated nature of the genital tract of a marsupial such as *Phascolarctos*, and one wonders if this has any connection with the frequent occurrence of serious disease in these organs. Drainage of fluid from uteri and cul-de-sac through the narrow lateral vaginae into the urogenital sinus could become inadequate if blockage occurred due to increased keratinization or impaction of cellular debris occurring during oestrous. This, in turn, could lead to grossly distended vaginae followed by infection of their contents. This phenomenon has been demonstrated experimentally in a closely related marsupial, *Trichosurus vulpecula*, after oestrogen administration (Bolliger 1946).

Of the 10 female koalas autopsied during the past three years, six showed gross lesions affecting the genital tract, mostly in the form of cysts. In four instances the genital pathology seemed to be one of the main contributing factors to the animal's death, and given sufficient time it seemed quite possible that the remaining two cystic tumours might also have ultimately caused death.

An uncomplicated form of unilateral cystic disease is represented by koala K58, which died of cryptococcosis of the brain. On opening the peritoneal cavity, a large cyst (5 by 3 cm) of the right ovary was discovered, which contained a brown fluid. On histological examination the tumour was found to resemble a serous cystadenoma as seen in the human female. The left ovary appeared to be normal.

In specimen K61 both ovaries were cystic and the tubes also contained cysts (Plate 2, Fig. 2). These tumours were also of the serous cystadenoma type. The

actual cause of death could not be determined. A large unilateral serous cystadenoma developed in specimen K53. Here the contents became infected and an abscess formed (Plate 3). Specimen K62 showed widespread infection of the genital tract. In this instance not only were the cysts infected, but the uteri and vaginal complex were also filled with pus (Plate 4). The infection from the left ovary spread through the tissues of the abdominal wall laterally and caudally and formed an abscess cavity extending as far as the iliac crest. In addition, there were numerous large tapeworms (*Prototaenia obesa*) in the small intestine.

The carcass of koala K64 was in a bad state of preservation when it came to autopsy. However, a large ovarian cyst containing chocolate-coloured fluid was present on the left side. On the right side an extensively infected cyst had perforated into the peritoneal cavity. The type of these cysts could not be determined with certainty because the epithelium was missing on the tissue specimen prepared for microscopy.

Finally, a bilateral ovarian tumour had developed in koala K55 which, on microscopic examination, proved to be a pseudomucinous cystadenoma. One of the large cysts had ruptured and the mucoid contents had escaped into the peritoneal cavity, resulting in widespread adhesions of coils of intestine which, in turn, must have led to fatal intestinal obstruction.

The outstanding points in these findings are the frequent occurrence of ovarian cysts and their marked tendency to become infected. In human females ovarian cysts very rarely become infected (Roques *et al.* 1959).

IV. Discussion

It has been pointed out that the koala of New South Wales is in danger of slow extinction because many of the animals die apparently at a comparatively early age (Bolliger and Backhouse 1960). The present series of autopsies seemed to confirm this impression, most koalas dying of a variety of diseases before reaching senility. This was particularly marked in the case of the females, which were prone to develop severe infections in the genital tract secondary to cysts in the ovaries and tubes. Furthermore, of three cases of cryptococcosis, all occurred in females. These diseases must have an adverse effect on the number of offspring, and, judging from the state of the pouch, one gained the impression that the majority of these females had never reared young.

The original purpose of this study was to throw some light on the cause of death of the koala, and particularly to look for unusual diseases which would be typical for the species. However, sepsis and pneumonia were relatively frequent causes of death besides a variety of other diseases. Outstanding among these were the frequent formation of ovarian cysts, which became infected in a number of cases, and cryptococcosis, so far not yet reported in other Australian animals.

Of course, it is realized that the number of koalas autopsied is too small to draw any general conclusions. The only corroborating evidence published is a short statement by McKenzie (1919) that the female native bear is prone to ovarian cysts. In addition, Mr. J. McNally, Victorian Department of Fisheries and

Game, informed us in a personal communication that in Victoria cystic conditions of the ovaries are often seen in the koala. McNally also noticed infestation with the cestode parasite *Prototaenia obesa*, which was seen once in our series in koala K62. He also observed necrotic areas in the liver. The latter could be related to the hepatitis and suppurative cholangitis as seen in three koalas in our series.

Post-mortem findings in Victoria together with our own seem to suggest that *Phascolarctos* suffers from diseases related to its anatomy and physiology. Reference has already been made to the anatomy of the female reproductive tract which is frequently afflicted with ovarian cysts which may become infected. Furthermore, the exclusive diet of eucalyptus leaves with their high essential oil content may pose unusual problems to hepatic activity and particularly detoxication (Hinks and Bolliger 1957), which may create pathological changes and lead to the frequent occurrence of liver insufficiency. However, in spite of these potential handicaps, it is hoped that koalas may still maintain their numbers in New South Wales since there is considerable public interest in this quaint marsupial and sanctuaries for its preservation are being extended.

V. Acknowledgments

We are deeply indebted to Sir Edward Hallstrom for enabling us to examine the koalas and to Dr. V. J. McGovern and Professor F. R. Magarey for their help. We also wish to thank the staff of the Department of Illustration, New Medical School, University of Sydney, for the photography.

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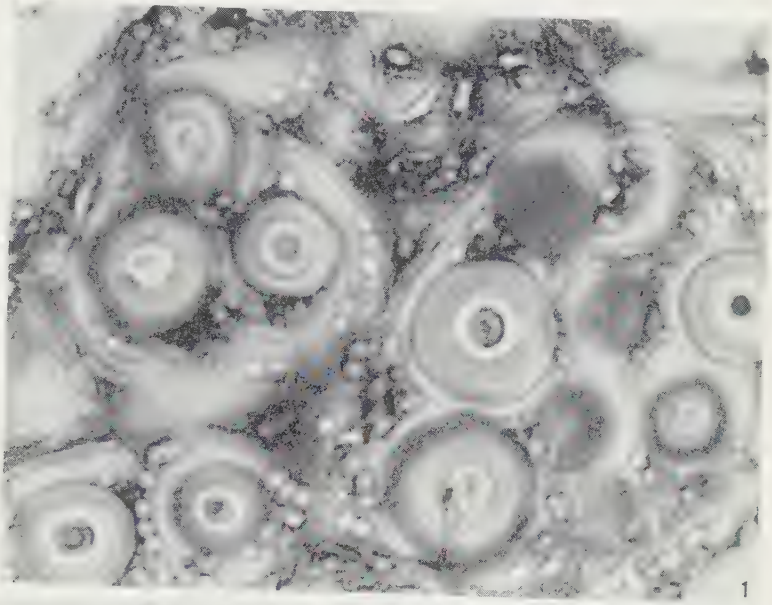
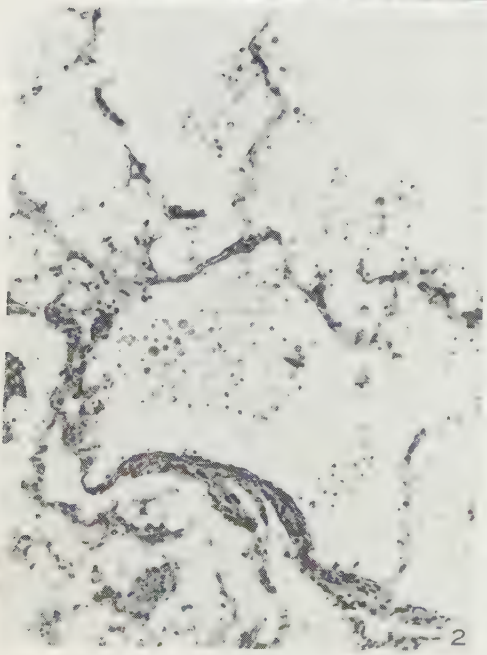
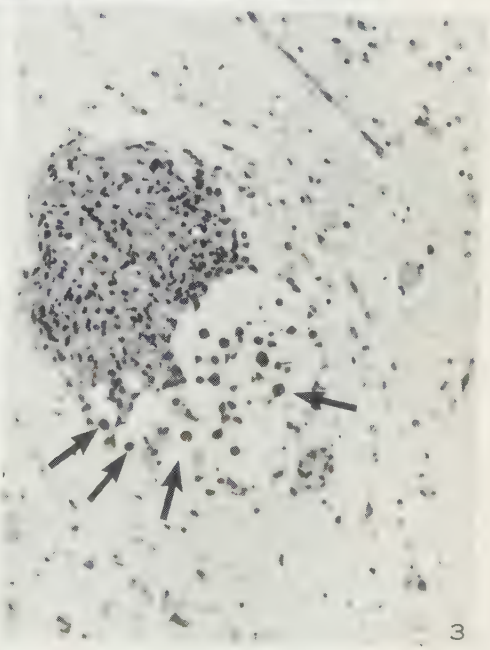
MORBIDITY AND MORTALITY IN THE KOALA







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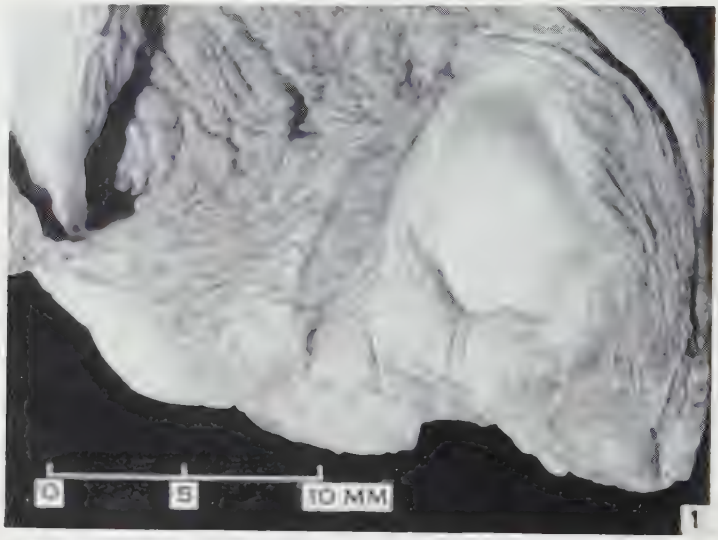

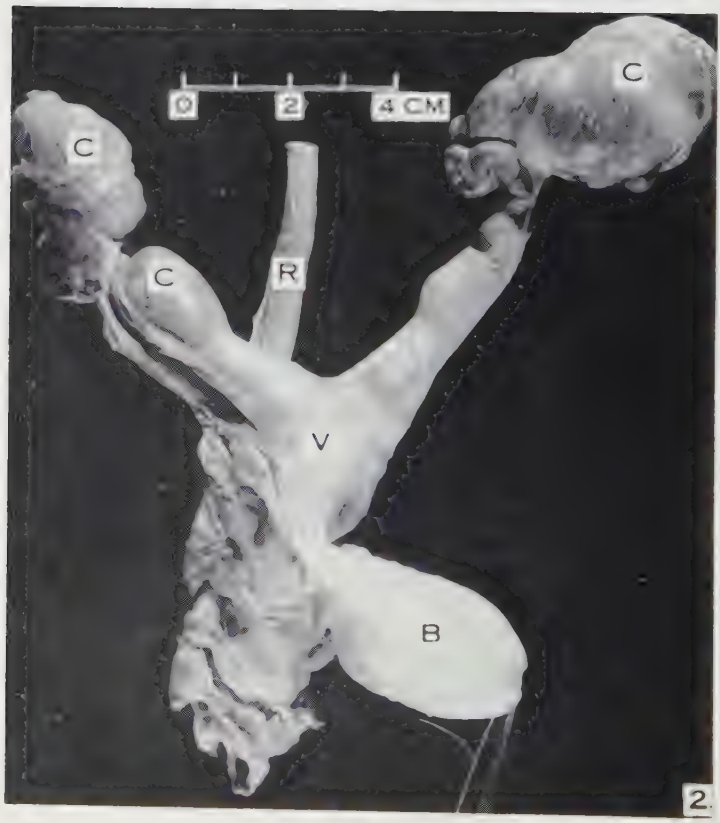

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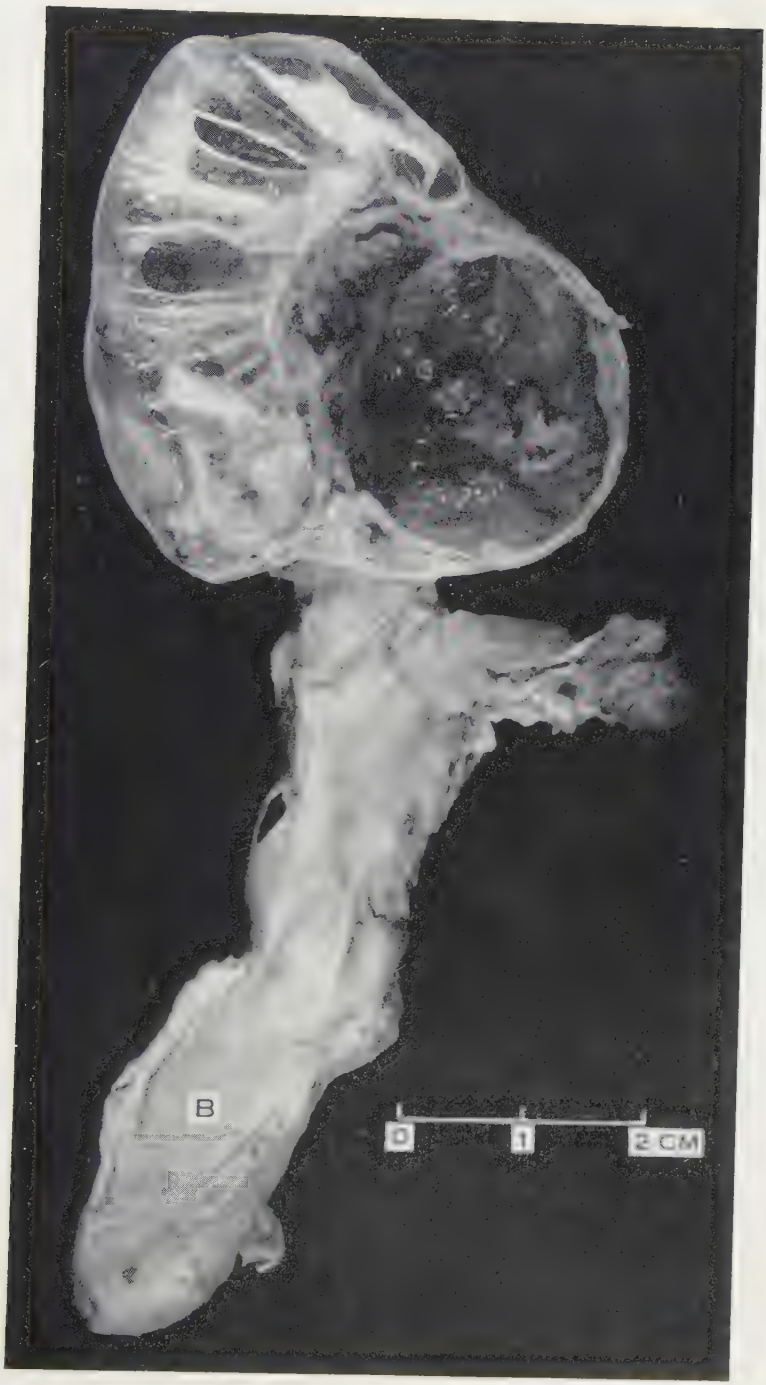

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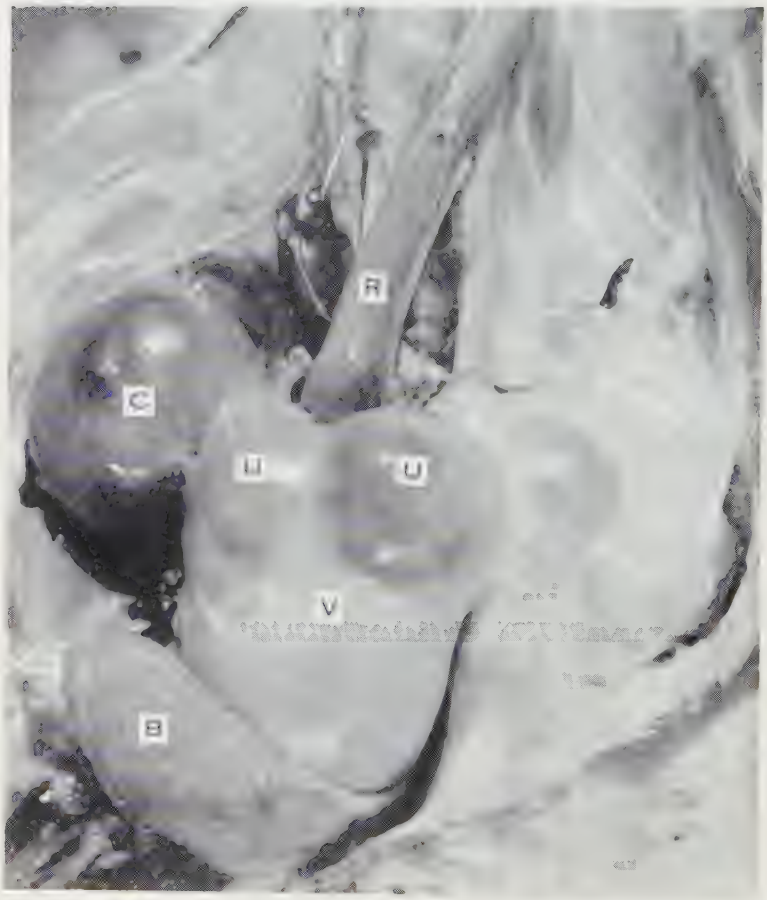

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EXPLANATION OF PLATES 1–4

PLATE 1

Fig. 1.—Koala K57. Cryptococcosis of lung. *Cryptococcus neoformans* proliferating in interstitial lung tissue. Haemotoxylin and eosin stain. Phase-contrast illumination. × *c.* 660.

Fig. 2.—Koala K57. Cryptococcosis of lung. Capsulated organisms occupying alveolar spaces and interstitial angles. Alveolar walls ruptured, forming larger confluent areas of invasion. Periodic acid–Schiff stain. × 140.

Fig. 3.—Koala K58. Section of brain showing granulomatous lesion and capsulated organisms. Arrows indicate cryptococci. Periodic acid–Schiff stain. × 140. See also Backhouse and Bolliger (1960).

PLATE 2

Fig. 1.—Koala K59. Cryptococcosis of lung. Nodule projecting from diaphragmatic surface of right lung.

Fig. 2.—Koala K61. Bilateral cystic ovaries and tubes. Histologically these tumours are serous cystadenomata. *B*, bladder; *C*, cysts; *R*, rectum; *V*, vaginal complex.

PLATE 3

Koala K53. Genital tract. The right ovary (bisected) has developed into a large cyst consisting of two compartments. The tumour which resembles histologically a serous cystadenoma became infected. *B*, bladder.

PLATE 4

Koala K62. Widespread infection of the genital tract, including ovarian cysts (*C*), uteri (*U*), and vaginal complex (*V*). *B*, bladder; *R*, rectum.

THE MITOTIC CHROMOSOMES OF MARSUPIALS AND THEIR BEARING ON TAXONOMY AND PHYLOGENY

By G. B. Sharman*

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Summary

Chromosome numbers of marsupials vary between $2n = 11$ ♂ 10 ♀ and $2n = 24$. Most species have 14 or 22 chromosomes. There is no evidence that polyploidy has occurred in marsupial evolution.

The Dasyuridae have 12 metacentric autosomes, a small metacentric *X*-chromosome and a very small *Y*-chromosome (20% of living species have been studied) and the chromosomes of *Myrmecobius fasciatus* are typically like those of other Dasyuridae.

The Peramelidae (30% of species have been studied) have chromosomes like the Dasyuridae except that *X*- and *Y*-chromosomes are much larger. The occurrence of similar chromosome numbers in Dasyuridae and Peramelidae is not necessarily evidence of affinity.

The chromosomes of the Phascolomidae are similar in number and morphology to those of the Peramelidae and the resemblances are, almost certainly, due to parallel evolution. The chromosomes of *Phascolarctos* are unlike those of any of the Phalangeridae and this genus might be just as easily grouped with the Phascolomidae.

The Phalangeridae have considerable chromosomal heterogeneity but less than 20% of species have been studied. Two species of *Cercaërtus* have 12 metacentric autosomes and small sex chromosomes like all members of the Dasyuridae. This suggests that the primitive phalangers may have retained the chromosome number and morphology of possible dasyurid ancestors but the resemblances may be due to parallel evolution of similar chromosome number and morphology in separate groups.

The chromosomes have been studied in more than 50% of Macropodinae. Cytological evidence suggests that *Thylogale* (3 species studied), *Petrogale* (2 species studied), and probably *Lagorchestes* (1 species studied), all with 22 chromosomes, are a related group. *Onychogalea unguifer,* with 20 chromosomes, may be derived from this group. There is no justification for the placing of *Thylogale billardierii* in the genus *Protemnodon. Lagostrophus fasciatus* has $2n = 24$ and its placement in a monotypic genus is justified. *Macropus major* and all species of *Protemnodon,* except *P. bicolor,* are a related group with 16 chromosomes. *M. robustus* is possibly included in this group. *M. rufus* has 20 chromosomes and should perhaps be placed in the separate genus *Megaleia. P. bicolor,* with 11 chromosomes in the male and 10 in the female, differs from all other species of *Protemnodon* and this genus, as at present constituted, may be diphyletic. The relationships of *P. bicolor* are unknown. *Setonix brachyurus* has 22, mostly metacentric, chromosomes and its affinities are at present unknown.

Three species of *Bettongia* (Potoroinae) have 22 chromosomes which are mostly metacentric. *Hypsiprymnodon moschatus* has 22 chromosomes which are mostly acrocentric. Both genera are very different cytologically from *Potorous tridactylus.*

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I. Introduction

The earliest reliable description of the chromosomes of a marsupial is that of Painter (1922) who showed that *Didelphis virginiana* had 22 chromosomes consisting of 10 pairs of rod-shaped (acrocentric) autosomes and an *X*- and a *Y*-chromosome in the male. Agar (1923) and Greenwood (1923) showed that Australian marsupials, like their American relatives, had relatively few large chromosomes. All of these authors were preceded by Benda (1906) whose figures of the chromosomes of *Perameles* (specific name not given) illustrated the large size of marsupial chromosomes. Benda's determination of 12 chromosomes is almost certainly incorrect — five species of the Peramelidae, including three species of *Perameles*, have 14 chromosomes (Table 1).

Agar (1923) described the sex bivalent of *Protemnodon bicolor* (= *Macropus ualabatus*) as being "attached to one of the autosomes" during meiosis, an observation which Matthey (1949) and Sharman, McIntosh, and Barber (1950) interpreted as evidence of a multiple sex-chromosome mechanism. Examination of this species during this investigation shows that *P. bicolor* has an XY_1Y_2 ♂ : XX ♀ sex-determining mechanism like that of *Potorous tridactylus* (Sharman and Barber 1952). All other species of marsupials so far studied have XY ♂ : XX ♀ sex-determining mechanisms. The sex chromosomes of a number of these species were described by Koller (1936) and McIntosh and Sharman (1953).

Darlington (1957) estimated that there are 47 Recent species of marsupials in New Guinea and adjacent islands and 119 in Australia. Some 10 or so species are common to both Australia and New Guinea so a reasonable total estimate is 160 Australasian species. Cabrera (1957) listed 69 Recent species of American marsupials. Chromosome counts published here double previous knowledge of marsupial cytology and bring the total number of reliable determinations for marsupials to 50 — some 22% of living species. All except three of the known chromosome numbers of marsupials apply to Australasian species 30% of which have had their chromosomes studied. The Australian family Macropodidae is exceptionally well known, there being reliable chromosome counts for over half the living species. According to Matthey (1958) reliable chromosome determinations have been made in 8% of the family Muridae and in 8% of all eutherian mammals. The Muridae is the largest mammalian family and, in the sense used by Matthey, corresponds to the superfamily Muroidea of Simpson (1945). White (1957*a*) estimated that some 0·4% of all animals have had their chromosomes counted. The marsupials are thus comparatively well known cytologically — perhaps better so than almost any other animal group.

This paper does not propose a new classification of marsupials based on their chromosome numbers but attempts to show how chromosome cytology may contribute to marsupial systematics. Ride (1957) has emphasized that the taxonomist needs data from many lines of investigation in order to clarify problems of animal relationships. One of these lines, comparative chromosome cytology, has so far been largely ignored by mammalian taxonomists — perhaps because study of mammal chromosomes was once a laborious procedure which gave rather doubtful results. However, many groups of insects are now well known

TABLE 1

CHROMOSOME NUMBERS OF MARSUPIALS

Species*	2*n*	Sex Chromosomes	Locality†	No. of Specimens Examined‡	Reference
DIDELPHIDAE					
Didelphis azarae Temminck (*D. paraguayensis*)	22	*XY/XX*	S. America		Saez (1931)
Didelphis marsupialis aurita Wied. (*D. aurita*)	22	*XY/XX*	S. America		Dreyfus and Campos (1941)
Didelphis marsupialis virginiana Kerr (*D. virginiana*)	22	*XY/XX*	N. America		Painter (1922)
Lutreolina crassicaudata Desmarest	22	*XY/XX*	S. America		Saez (1938)
DASYURIDAE					
Dasyurinae					
Dasyurus hallucatus Gould	14	*XY/XX*	Woodstock and Mt. Herbert, N.W. Aust.	4	This paper
Dasyurus maculatus Kerr	14	*XY/XX*	———		Greenwood (1923); Koller (1936)
Dasyurus quoll Zimmerman (*D. viverrinus*)	14	*XY/XX*	———		Drummond (1938)
Sarcophilus harrisii Boitard (*S. ursinus*)	14	*XY/XX*	Tasmania		Greenwood (1923); Koller (1936); McIntosh and Sharman (1953)
Phascogalinae					
Antechinus flavipes Waterhouse	14	§	Upper Allyn, N.S.W.	1	This paper
Sminthopsis crassicaudata Gould	14	*XY/XX*	S.W. Aust., Cowell, S. Aust.	4	This paper
Sminthopsis macrura Gould	14	*XY/XX*	Oodnadatta, S. Aust.	1	This paper
Myrmecobiinae					
Myrmecobius fasciatus Waterhouse	14	*XY/XX*	Dryandra and Albany Highway, 58 miles S. of Perth, S.W. Aust.	3	This paper

* When the chromosomes of a species or subspecies were described under a different name this is given in brackets.
† Localities are general for species previously described; a dash indicates that no locality was given.
‡ The numbers of specimens refer only to work reported here.
§ Female material only studied.

TABLE 1 (*Continued*)

Species	2n	Sex Chromosomes	Locality	No. of Specimens Examined	Reference
PERAMELIDAE					
Isoodon macrourus Gould	14	*XY/XX*	Tooloom, N.S.W.	1	This paper
Isoodon obesulus Shaw	14	*XY/XX*	———		Drummond (1933)
			Tasmania		McIntosh and Sharman (1953)
Perameles bouganvillei Quoy & Gaimard	14	*XY/XX*	Dorre I., W. Aust.	1	This paper
Perameles gunnii Gray	14	*XY/XX*	Tasmania		McIntosh and Sharman (1953)
Perameles nasuta Geoffroy	14	*XY/XX*	Upper Allyn, N.S.W.	2	This paper
PHALANGERIDAE					
Phalangerinae					
Cercaërtus concinnus Gould	14	*XY/XX*	Bordertown and Keith, S. Aust.	2	This paper
Cercaërtus lepidus Thomas	14	*XY/XX*	Westbury, Tas.	2	This paper
Petaurus breviceps Waterhouse	22	*XY/XX*	———		Drummond (1933)
Trichosurus vulpecula Kerr	20	*XY/XX*	Queensland and Victoria		Altmann and Ellery (1925); Koller (1936)
Trichosurus caninus Ogilby	20	*XY/XX*	Victoria	1	This paper
Phascolarctinae					
Phascolarctos cinereus Goldfuss	16	*XY/XX*	Victoria		Greenwood (1923); Koller (1936)
Pseudocheirus peregrinus Boddaert	20	*XY/XX*	———		Altmann and Ellery (1925); Koller (1936)
Schoinobates volans Kerr (*Petauroides volans*)	22	*XY/XX*	———		Agar (1923)
PHASCOLOMIDAE					
Lasiorhinus latifrons Owen	14	*XY/XX*	Blanche Town and Nullarbor Station, S. Aust.	6	This paper
Phascolomis ursinus Shaw	14	*XY/XX*	———		Altmann and Ellery (1925)
			Canberra; Rendelsham, S. Aust.	2	This paper

TABLE 1 (*Continued*)

Species	2*n*	Sex Chromosomes	Locality	No. of Specimens Examined	Reference
MACROPODIDAE					
Macropodinae					
Lagorchestes hirsutus Gould	22	*XY/XX*	Dorre I., W. Aust.	1	This paper
Lagostrophus fasciatus Peron & Lesueur	24	*XY/XX*	Bernier and Dorre Is., W. Aust.	3	This paper
Macropus major fuliginosus Gould	16	*XY/XX*	Kangaroo I., S. Aust.	3	This paper
Macropus major major Shaw	16	*XY/XX*	Princetown, Vic.	2	This paper
Macropus major melanops Gould	16	*XY/XX*	Blanche Town, S. Aust.	2	This paper
Macropus major ocydromus Gould (*M. ocydromus*)	16	*XY/XX*	South-western Australia		McIntosh and Sharman (1953)
Macropus major tasmaniensis Le Souef (*M. tasmaniensis*)	16	*XY/XX*	Tasmania		McIntosh and Sharman (1953)
Macropus robustus Gould	16	*XY/XX*	Big Bell, W. Aust.; Hamersley Range, N.W. Aust.	4	This paper
Macropus rufus Desmarest (*Megaleia rufa*)	20	*XY/XX*	New South Wales		McIntosh and Sharman (1953)
			Tambrey Station, N.W. Aust.	2	This paper
Onychogalea unguifer Gould	20	*XY/XX*	26 miles W. of Fitzroy Crossing, N.W. Aust.	1	This paper
Petrogale penicillata pearsoni Thomas	22	*XY/XX*	Pearson I., S. Aust.	6	This paper
Petrogale rothschildi Thomas	22	*XY/XX*	Woodstock, N.W. Aust.	2	This paper
Protemnodon agilis Gould	16	*XY/XX*	60 miles W. of Fitzroy Crossing, N.W. Aust.	3	This paper
Protemnodon bicolor Desmarest	11♂, 10♀	XY_1Y_2/XX	Peterborough, Vic.	4	This paper
Protemnodon dorsalis Gray	16	*XY/XX*	Bonalbo, N.S.W.	1	This paper

TABLE 1 (*Continued*)

Species	2*n*	Sex Chromosomes	Locality	No. of Specimens Examined	Reference
Protemnodon eugenii Desmarest	16	*XY/XX*	South-western Australia		Sharman (1954)
Protemnodon irma Jourdan	16	*XY/XX*	40 miles W. of Ravensthorpe, S.W. Aust.	2	This paper
Protemnodon parryi Bennett (*Macropus parryi*)	16	*XY/XX*	Eastern Australia		Matthey (1934)
Protemnodon rufogrisea Desmarest (*Wallabia rufogrisea*)	16	*XY/XX*	Tasmania		McIntosh and Sharman (1953)
			Princetown, Vic.	2	This paper
Setonix brachyurus Quoy & Gaimard	22	*XY/XX*	South-western Australia		Drummond (1933); Sharman (1954)
Thylogale billardierii Desmarest	22	*XY/XX*	Tasmania		McIntosh and Sharman (1953)
			Nile, Tas.	1	This paper
Thylogale stigmatica Gould	22	*XY/XX*	Upper Allyn and Mt. Lindesay, N.S.W.	2	This paper
Thylogale thetis Lesson	22	*XY/XX*	Upper Allyn, N.S.W.	1	This paper
Potoroinae					
Bettongia cuniculus Ogilby	22	*XY/XX*	Tasmania		McIntosh and Sharman (1953)
Bettongia lesueur Quoy & Gaimard	22	*XY/XX*	———		Drummond (1933)
			Bernier and Dorre Is., W. Aust.	2	This paper
Bettongia penicillata Gray	22	*XY/XX*	Dryandra, Pingelly, S.W. Aust.	5	This paper
Hypsiprymnodon moschatus Ramsay	22	*XY/XX*	Innisfail, Qld.	1	This paper
Potorous tridactylus Kerr	13♂, 12♀	XY_1Y_2/XX	Tasmania		McIntosh and Sharman (1953)

cytologically and White (1957*b*) has shown how the information may contribute to taxonomy. The cytologists viewpoint has been admirably expressed by White who stated: "Cytogenetics is not an automatic solution to all systematic problems, neither is it a final court of appeal in all difficult situations. But in suitable cases it can provide critical evidence of a unique kind."

II. Materials and Methods

Chromosome counts were made from Feulgen squash preparations of bone marrow or testis or both. The colchicine hypotonic citrate squash technique of

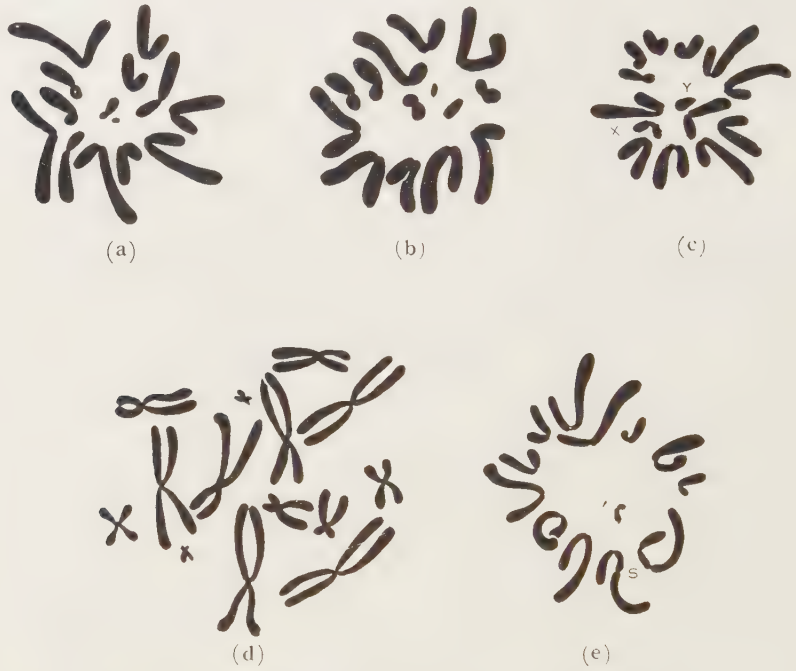

Fig. 1.—Metaphase of mitosis in marsupials, with 14 chromosomes. (*a*) *Sminthopsis crassicaudata,* spermatogonial mitosis with *X*- and *Y*-chromosomes at the centre of the plate; (*b*) *Myrmecobius fasciatus,* spermatogonial mitosis — the two smallest chromosomes are *X* and *Y*; (*c*) *Perameles nasuta,* spermatogonial mitosis; (*d*) *Antechinus flavipes,* from bone marrow cell of female pretreated with colchicine and sodium citrate — the chromatids are separated except at the centromeres; (*e*) *Cercaërtus lepidus,* spermatogonial mitosis with *X*- and *Y*-chromosomes at the centre of the plate. *S*, secondary constrictions.

Ford and Hamerton (1956) gave good results but could not be used in most cases since the specimens were shot or trapped in the field. Material collected in the field was treated as previously described (Sharman and Barber 1952; McIntosh and Sharman 1953).

All drawings were made, with the use of a camera lucida, at an initial magnification of × 5000. They are reproduced here at a magnification of × 2500. In drawing the preparations overlapping chromosomes were separated for the sake of clarity.

A complete list of chromosome numbers of marsupials, including new counts determined in this investigation, is given in Table 1. Earlier chromosome counts, since shown to be incorrect, and those considered to be wrong (e.g. Vejdovsky's (1926) determination of $2n = 17$ for the male of *Petrogale* sp.) are omitted.

III. New Chromosome Determinations

(*a*) *Dasyuridae*

Males of *Sminthopsis crassicaudata* (Fig. 1) and *Dasyurus hallucatus* (Fig. 2) have six pairs of metacentric autosomes, a small metacentric *X*-chromosome, and a very small *Y*-chromosome. The *X*-chromosome is smaller than the

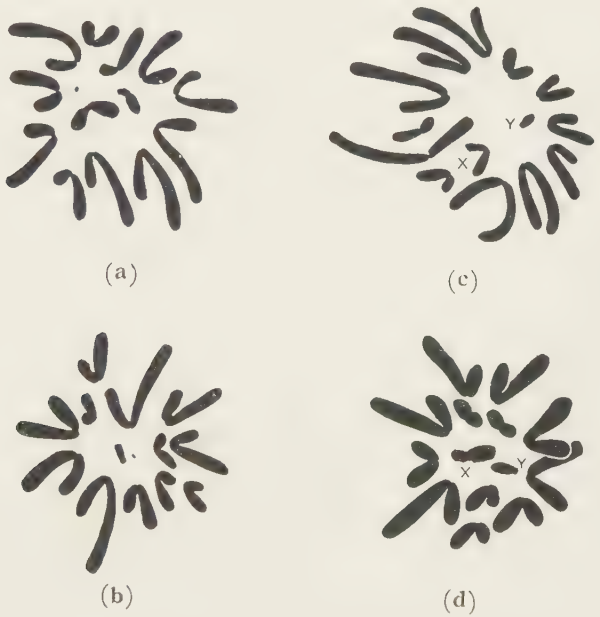

Fig. 2.—Metaphase of spermatogonial mitosis in unrelated marsupials with the same chromosome number and morphology. (*a*) *Dasyurus hallucatus* (Dasyuridae) and (*b*) *Cercaërtus concinnus* (Phalangeridae) — both have 12 metacentric autosomes, a small *X*-chromosome, and a minute *Y*-chromosome; (*c*) *Lasiorhinus latifrons* (Phascolomidae) and (*d*) *Isoodon macrourus* (Peramelidae) — both have 12 metacentric autosomes and an *X*-chromosome about equal in size to the smallest autosome. The difference in the thickness of the chromosomes between (*c*) and (*d*) is not of special significance as this varies in different types of spermatogonia.

smallest autosome. A single juvenile male of *S. macrura*, in which only somatic mitoses were studied, agrees in chromosome number and morphology with *S. crassicaudata*. The chromosome number and morphology in *Myrmecobius fasciatus* (Fig. 1) agrees closely with that of Dasyuridae described here and by previous authors (Greenwood 1923; Koller 1936; Drummond 1938). In this species the *X*-chromosome is also metacentric (although this is not always obvious at the

metaphase stage in spermatogonial mitoses) and smaller than the smallest autosome. Material of *Antechinus flavipes* was limited to a single female in which dividing bone marrow cells (Fig. 1) were studied. There are 14 chromosomes, the largest 12 being comparable in size and shape to the various autosomes in the other species. It is assumed that the two smallest, metacentric chromosomes are the sex chromosomes and that the sex-determining mechanism is of the XY ♂ : XX ♀ type as in other Dasyuridae. Until the male is studied this cannot be decided with certainty.

(*b*) *Peramelidae*

Perameles bouganvillei, *P. nasuta*, and *Isoodon macrourus* have the same chromosome number and morphology as have previously described members of this family (Drummond 1933; McIntosh and Sharman 1953). There are six pairs of metacentric autosomes and a heteromorphic pair of sex chromosomes in the male (Figs. 1 and 2). The X-chromosome differs at once from that of Dasyuridae in that it is equal to, or exceeds in size, the smallest autosome. The Y-chromosome is also much larger than in any of the Dasyuridae.

In an earlier paper (McIntosh and Sharman 1953) the chromosomes of *I. obesulus* were described from material collected in Queensland and Tasmania. The Queensland specimens, obtained by Dr. I. M. Mackerras, are now known to have been *I. macrourus*, not *I. obesulus* (see Mackerras and Mackerras 1960). As stated above the chromosome complements of the two species are very similar.

(*c*) *Phalangeridae*

Cercaërtus lepidus (Fig. 1) and *C. concinnus* (Fig. 2) both have 14 chromosomes. All the autosomes have near median centromeres and the sex chromosomes are of small size. As in the Dasyuridae the X-chromosome is smaller than the smallest autosome and the Y is minute. The chromosome complement in two species of *Cercaërtus* thus shows a remarkable resemblance to that of the Dasyuridae. One pair of autosomes in *C. lepidus* has secondary constrictions (Fig. 1) but no such constrictions were seen in *C. concinnus*. In other respects the chromosome complements of the two species appear identical.

A brief cytological examination was made of *Trichosurus caninus*. Like *T. vulpecula* (Altmann and Ellery 1925; Koller 1936) this species has 20 chromosomes. The cytology of *Trichosurus* is at present being examined in detail by Mr. J. A. Thomson at the University of Melbourne.

(*d*) *Phascolomidae*

Altmann and Ellery's (1925) determination of $2n = 14$ for *Phascolomis ursinus* was confirmed. As in the material studied by these authors spermatogonial mitoses were absent but later stages of spermatogenesis were abundant. In *Lasiorhinus latifrons* the testes are scrotal and active in the breeding season and inguinal and dormant at other times. Spermatogonial mitoses are abundant before the breeding season when the testes are beginning to descend into the scrotum

and absent at later times. The same condition may hold in *Phascolomis* thus explaining the absence of spermatogonial mitoses in animals in which later stages of spermatogenesis are abundant.

L. latifrons has 14 chromosomes. The autosomes all have near median centromeres and the *X*-chromosome is about equal in size to the smallest autosome. The chromosome complement is thus very like that of the Peramelidae (Fig. 2).

(*e*) *Macropodidae*

Lagostrophus fasciatus has 24 chromosomes (Fig. 3) and is the only marsupial, thus far studied, in which this chromosome number occurs. One pair

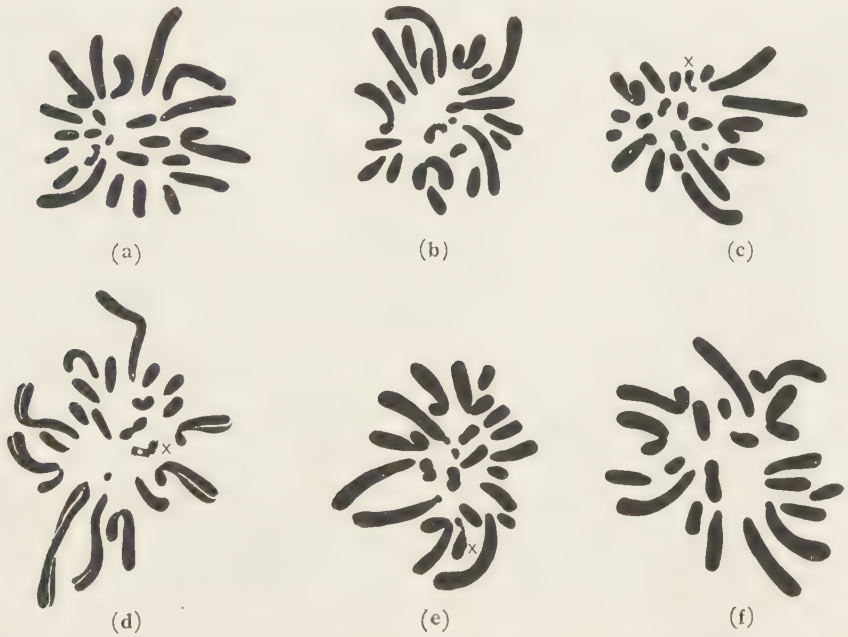

Fig. 3.—Metaphase of spermatogonial mitosis in macropod marsupials. (*a*) *Lagostrophus fasciatus*; (*b*) *Lagorchestes hirsutus* — the *X*- and *Y*-chromosomes are at the centre of the plate; (*c*) *Thylogale thetis*; (*d*) *Thylogale stigmatica*; (*e*) *Petrogale rothschildi*; (*f*) *Onychogalea unguifer* — the chromosomes in the centre of the plate at 6 o'clock and 3 o'clock may be the *X* and *Y*. Note the resemblances between (*c*), (*d*), and (*e*) all of which show 22 chromosomes and an *X*-chromosome with an undercondensed segment near the centromere.

of the large autosomes are metacentric and the remainder acrocentric. The *X*-chromosome is metacentric.

Lagorchestes hirsutus, Petrogale penicillata, P. rothschildi, Thylogale stigmatica, and *T. thetis* have 22 chromosomes. The chromosome morphology of all species is similar (Fig. 3). There are two large pairs of acrocentric autosomes, two large pairs of metacentrics, and six pairs of smaller autosomes. In both species of *Thylogale,* both species of *Petrogale,* and probably also in *Lagorchestes hirsutus,* one of the smaller autosomal pairs are metacentric. In all of these species,

except *L. hirsutus*, the *X*-chromosome has a pronounced undercondensed segment near the centromere at metaphase of spermatogonial mitosis. In prophase cells of *Petrogale* this region is completely, or almost completely, non-staining with Feulgen reagent and the *X*-chromosome looks like two closely apposed small chromosomes. McIntosh and Sharman (1953) reported a similar condition in the *X*-chromosome of *Thylogale billardierii*.

Onychogalea unguifer has 20 chromosomes (Fig. 3). Two of the larger autosomal pairs are metacentric as in *Thylogale* and *Petrogale*. The sex chromosomes of *Onychogalea* are rather different from those of all other marsupials described here. Generally speaking the sex chromosomes of marsupials are obvious at metaphase stages of mitosis in the male because the *X* is undercondensed, and

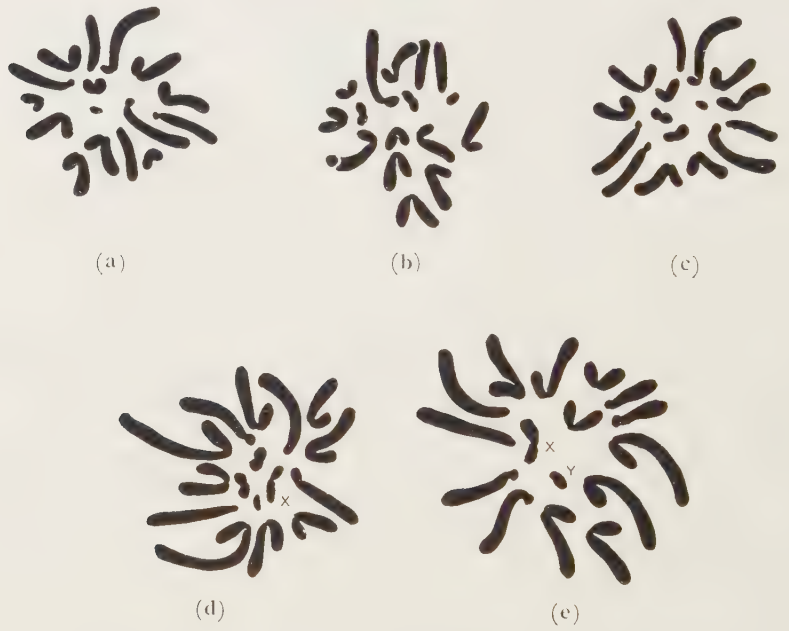

Fig. 4.—Metaphase of spermatogonial mitosis in macropod marsupials with 16 chromosomes. (*a*) *Protemnodon agilis*; (*b*) *P. dorsalis*; (*c*) *P. irma*; (*d*) *Macropus major fuliginosus*; (*e*) *M. robustus*.

the *Y* very small, relative to the autosomes. Two chromosomes without equal-sized homologues appear in metaphase preparations of *Onychogalea*. These are a medium-sized chromosome, with a submedian constriction which is presumably the centromere constriction, and a rod-shaped chromosome rather larger than the smallest autosome. The larger of these two sometimes exhibits a slight undercondensation at mitotic metaphase but, in general, neither of these, nor any others in the set, exhibit differential condensation during mitosis. At first division of meiosis one bivalent is precociously condensed during prophase stages as are the *XY* bivalents of other marsupials. At first metaphase one of the 10 bivalents, which is assumed to be the *XY*, is decidedly composed of unequal-sized chromo-

somes but is rather different in shape and behaviour from the *XY* bivalents of other marsupials. This bivalent, which is larger than the *XY* bivalents of other marsupials, does not exhibit precocious segregation at early first anaphase and does not lag at late anaphase stages as the *X* and *Y* normally do (see McIntosh and Sharman 1953). Moreover, the sex chromosomes do not appear to lag at late second anaphase stages as do those of the marsupials described by McIntosh and Sharman.

Protemnodon agilis, P. dorsalis, P. irma, Macropus major, and *M. robustus* have 16 chromosomes (Fig. 4). In all of these species there is one pair of

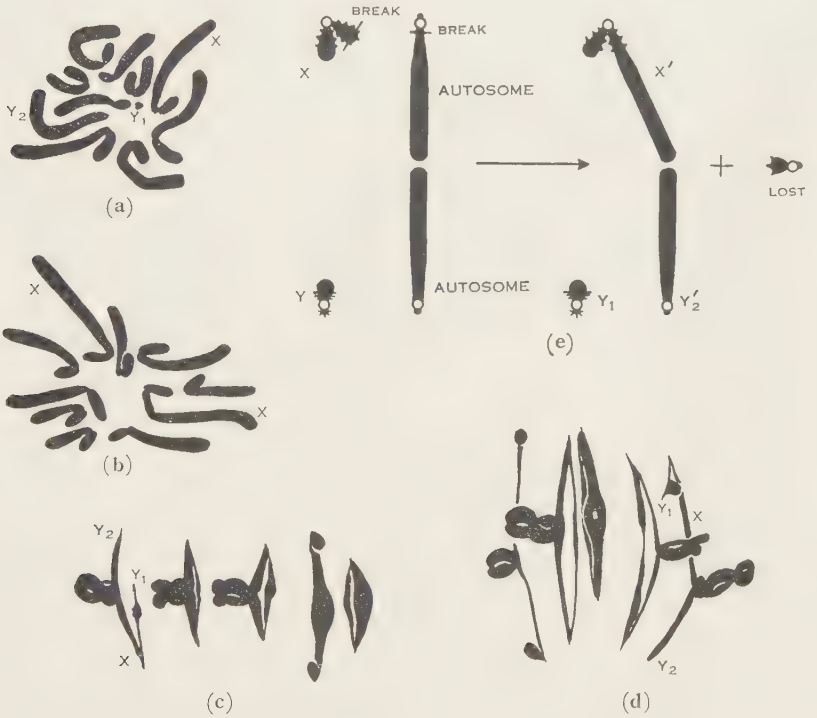

Fig. 5.—*Protemnodon bicolor*: (*a*) metaphase of spermatogonial mitosis; (*b*) metaphase of mitosis from bone marrow cell of female; (*c*) first metaphase of meiosis; (*d*) "prestretch" stage in which XY_1 portion of trivalent has not yet fully opened out on the spindle; (*e*) the origin of the XY_1Y_2 system from an ancestral *XY* system. Terminology as in White (1957*a*). Chromosome regions from the ancestral *X* shown with a wavy outline; differential segment of the *Y* shown with a fringed outline. *Break*, positions of chromosome breaks. For further explanation see text.

acrocentric autosomes and the remaining autosomes are V- or J-shaped. The short arms of some of the J-shaped chromosomes are minute so it becomes a matter of some doubt as to whether they should be described as acrocentric or metacentric. The chromosomes of *M. robustus* differ most from the remainder. In this species the smallest (V-shaped) pair of autosomes are rather larger than in the other species and equal, or slightly exceed, the *X*-chromosome in size.

In *M. major*, and the three species of *Protemnodon*, the *X*-chromosome is larger than the smallest autosome.

P. bicolor differs from all other species of its genus. There are 11 chromosomes in the male and 10 in the female (Fig. 5). The male complement consists of four pairs of metacentric chromosomes, a large metacentric chromosome without a similar homologue (X), a single large acrocentric chromosome (Y_2), and a very small chromosome (Y_1). At meiosis these last three chromosomes form a trivalent. The sex-determining mechanism of *P. bicolor* is therefore of the XY_1Y_2♂ : XX♀ type as in the marsupial *Potorous tridactylus* (Sharman and Barber 1952) and in the insectivore *Sorex araneus* (Sharman 1956). The large metacentric *X*-chromosome is composed of chromatin of two types which, in the male at least, condense differently at both mitosis and meiosis. The short arm and part of the long arm near the centromere, which together equal in size the metacentric *X*-chromosome of other species of *Protemnodon*, are undercondensed at the metaphase of

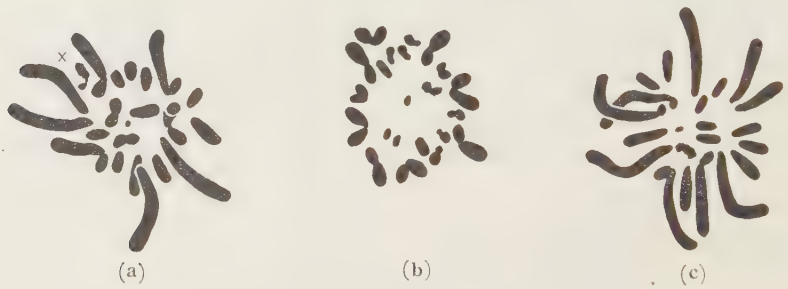

Fig. 6.—Metaphase of spermatogonial mitosis in marsupials with the same chromosome number ($2n = 22$) but different chromosome morphology. (*a*) *Petrogale penicillata*; (*b*) *Bettongia penicillata*; (*c*) *Hypsiprymnodon moschatus*. The shortness of the chromosomes in (*b*) is not of special significance as the contraction of metaphase chromosomes varies in different types of spermatogonia.

spermatogonial mitosis and overcondensed at the meiotic prophase. This is the typical behaviour of the *X*-chromosomes of other marsupials. The remainder of the *X*-chromosome (nearly all the long arm) and the long acrocentric chromosome (Y_2) behave, in both mitosis and meiosis, typically like the autosomes. The small chromosome of the male (Y_1) is exactly like the *Y*-chromosome of many other marsupials. It is assumed therefore that a pair of acrocentric autosomes have been incorporated into the sex-chromosome mechanism, the compound *X*-chromosome having been produced by what White (1957*a*) calls a "tandem fusion". Figure 5(*e*) shows the origin of the multiple system using White's (1957*a*) terminology in which "*X*′ and *Y*′ represent 'neo' sex chromosomes, i.e. ones containing some material of recent autosomal origin or consisting entirely of such material". A tandem fusion between a metacentric *X*-chromosome and an acrocentric autosome has also apparently been responsible for the formation of the XY_1Y_2♂ : XX♀ systems in *P. tridactylus* (Sharman and Barber 1952) and *S. araneus* (Sharman 1956).

Bettongia lesueur has 22 chromosomes. These were illustrated and described by Drummond (1933) whose account is confirmed. The chromosomes of *B. lesueur* are essentially like those of *B.cuniculus* which also has 22 chromosomes (McIntosh and Sharman 1953). Drummond (1933) stated the *B. penicillata* had 28 chromosomes, a finding that cannot be confirmed in specimens of this species from south-western Australia. Five specimens of *B. penicillata* from this region had 22 chromosomes (Fig. 6) alike in morphology to the chromosomes of *B. cuniculus* and *B. lesueur*. *B. penicillata* has a more or less coastal distribution, extending over some 3000 or more miles, from north-eastern Queensland to south-western Australia. It is perhaps possible that more than one species is represented in the animals referred to *B. penicillata* or alternatively this species may exhibit cytological polymorphism. Natural populations of animals polymorphic for centric fusions are known (see White 1957*a*) although Matthey (1958) states that the only certain case for mammals occurs in the insectivore *S. araneus* (Sharman 1956; Ford, Hamerton, and Sharman 1957). *B. penicillata* may, however, have a similar kind of cytological polymorphism to that found in *Sorex* since Drummond's (1933) clear illustrations indicate a much higher proportion of acrocentric chromosomes than are found in the material studied here.*

Hypsiprymnodon moschatus, like the three species of *Bettongia*, has 22 chromosomes but *Bettongia* has many metacentric chromosomes and *Hypsiprymnodon* has mostly acrocentrics (Fig. 6).

IV. The Relationships of Marsupials as Expressed by Chromosome Number and Morphology

(*a*) *General*

In eutherian mammals chromosome numbers vary between 17 and 78 and the average for 240 species is 46·79. There is a normal distribution of chromosome numbers with a modal number of 48. Not only the Eutheria as a whole but the most extensively studied family (Muridae), several subfamilies, and some genera have a normal distribution of chromosome numbers with a mode of 48. Matthey (1958) concluded that the eutherian mammals have evolved from a "pool" of species possessing 40–56 chromosomes and that the modal number of 48 is to be regarded as primitive. Species possessing less than 40 or more than 56 chromosomes

* Since this paper was submitted the chromosomes of *Aepyprymnus rufescens* from Richmond Range State Forest, N.S.W., have been studied. This species has between 28 and 32 chromosomes. The material studied by Drummond, and referred to *B. penicillata*, almost certainly came from Eidsvold, Qld. The identification was made by the late Dr. T. L. Bancroft and only the fixed testis was forwarded to Melbourne University where it was studied by Drummond (personal communication). Finlayson (1931) collected mammals in the Dawson Valley, Qld. (less than 100 miles from Eidsvold and in country of similar type) in the summer of 1928–29. He found *A. rufescens* to be common but he was unable to find *B. penicillata* which has probably been taken only once in central coastal Queensland — by Lumholtz during his expedition of 1880–84. It is thus probable that Drummond's count was based on wrongly identified material and should have applied to *A. rufescens*.

Matthey presumably regarded as evolutionary "dead ends" for he stated (p. 291): "Il en résulte que les grandes mutations chromosomiques (inversions péricentriques, fusions centriques) qui conduisent aux classes extrêmes sont postérieures à la différenciation générique et que leur valeur évolutive est très faible, pour ne pas dire nulle."

In marsupials, on the other hand, diploid chromosome numbers vary between 11♂ : 10♀ and 24 (Fig. 7). The upper limit is 28 if Drummond's (1933) count for *B. penicillata* is included. The average number of chromosomes in marsupials, to the nearest whole number, is 18 but no marsupial has 18 chromosomes. There is a bimodal distribution of chromosome numbers with peaks at 14 and 22.

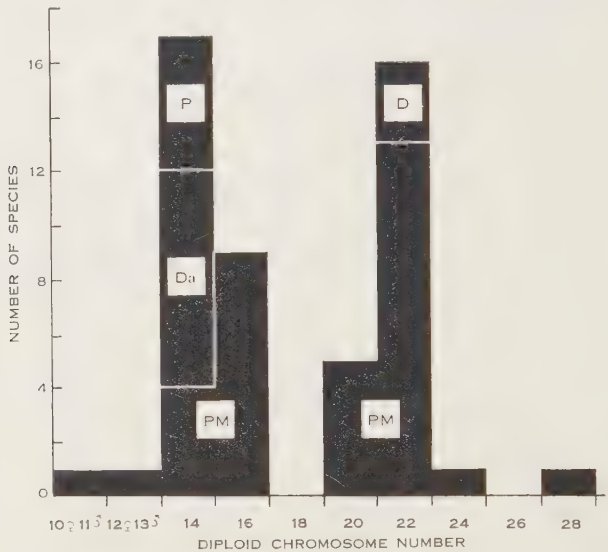

Fig. 7.—Histogram of chromosome numbers of marsupials, divided to show the numbers of species studied in each of the major groups. Drummond's (1933) count of 28 chromosomes for *Bettongia penicillata* is included as well as the count of $2n = 22$ obtained for the same species in this investigation (see text). *D*, Didelphidae; *Da*, Dasyuridae; *P*, Peramelidae; *PM*, Phalangeridae, Phascolomidae, and Macropodidae (superfamily Phalangeroidea).

Marsupials thus differ from eutherian mammals not only in possessing fewer chromosomes but in having a bimodal, instead of a unimodal, distribution of chromosome numbers.

As in eutherian mammals (Matthey 1958 and earlier papers) there is no evidence that polyploidy has occurred in marsupial evolution. White (1957*a*) has concluded that, contrary to the beliefs of several authors, polyploidy has not been important in the evolution of any group of bisexual animals.

On the analogy of Matthey's (1958) conclusion that the modal number of 48 is the primitive number for the Eutheria, either of the modal numbers 14 or 22 could presumably be regarded as the primitive number for marsupials. The

Australian groups are considered to be derived from didelphid-like ancestors modern forms of which have 22 chromosomes. Some Phalangeridae and many Macropodidae, including *Hypsiprymnodon* which is a primitive form in some respects, also have 22 chromosomes but this is almost the upper limit of marsupial chromosome numbers. Evolution in marsupials may thus have been accompanied by general reduction of chromosome numbers as we earlier suggested for other reasons (Sharman and Barber 1952). Matthey (1958), however, concluded that in the Eutheria evolution towards higher chromosome numbers had happened as frequently as evolution towards lower numbers.

(*b*) *Marsupial Families with Uniform Chromosome Numbers — Didelphidae, Dasyuridae, Peramelidae, and Phascolomidae*

The marsupial families Didelphidae, Dasyuridae, and Peramelidae are cytologically distinguishable from each other but the chromosome number and morphology are alike in all species of each family thus far studied. Admittedly only about 4% of the Didelphidae have been studied but all have 20 acrocentric autosomes, a metacentric *X*-chromosome, and a small *Y*-chromosome. Chromosome numbers are known in about 20% of Dasyuridae and all of these have 12 metacentric autosomes, a small metacentric *X*-chromosome, and a very small *Y*-chromosome. The Peramelidae are like the Dasysuridae except that the *X*- and *Y*-chromosomes are much larger. About 30% of the known species have been studied. The occurrence of similar chromosome numbers in the Dasyuridae and Peramelidae is not necessarily evidence of relationship, as we earlier assumed (McIntosh and Sharman 1953), since representatives of four families have now been shown to have 14 chromosomes (Table 1). Because of the extremely uniform chromosome complements found in the Dasysuridae and Peramelidae it is unlikely that any taxonomic inferences can be made from a study of the chromosomes. The aberrant *Myrmecobius fasciatus* has chromosomes typically like those of other Dasyuridae and there is no cytological evidence for its placement in a separate family as urged by Troughton (1959). Calaby (1960) considered *Myrmecobius* to be an aberrant member of the Dasyuridae and mentioned several of its dasyure-like features.

The chromosome complements of the wombats *Lasiorhinus latifrons* and *Phascolomis ursinus* are very like those of the Peramelidae (Fig. 2). It is unlikely that this is evidence of near relationship so the resemblances are presumed to be due to parallel development of the same chromosome number and morphology in taxonomically widely separated groups. Sonntag (1923) drew attention to the anatomical similarities between the wombats, which have $2n = 14$, and the koala (*Phascolarctos*) which has $2n = 16$. Besides the differences in number there are, to judge from published figures of koala chromosomes (Greenwood 1923; Koller 1936), differences in chromosome morphology. The koala is also rather different in chromosome number and morphology from the rest of the Phalangeridae, with which it is included in Simpson's (1945) classification, and might just as easily be included in the same family as the wombats as Sonntag (followed by Troughton 1959) advocated. Besides the similarities listed by Sonntag *Phascolarctos* and

Phascolomis share in common a similar type of placenta (Hill 1949) which has so far been found in no other marsupial.

The occurrence of whole families of marsupials characterized by constant chromosome numbers contrasts with the condition found in the Eutheria in which Matthey (1958) lists no family in which more than three species have been studied which has a constant chromosome number.

(*c*) *Marsupial Families with a Range of Chromosome Numbers — Phalangeridae and Macropodidae*

The family Phalangeridae, unlike the preceding families, has considerable cytological heterogeneity but less than 20% of the species have had their chromosomes studied and few, if any, inferences about relationships within the group can yet be drawn. Two species of *Cercaërtus* have the same number of chromosomes as have all species of Dasyuridae and the chromosome morphology is also strikingly similar (Fig. 2). While it is unlikely that the similarities between the chromosomes of *Cercaërtus* and the Dasyuridae are evidence of near relationship it must be borne in mind that some species of *Cercaërtus* are amongst the most generalized of the subfamily Phalangerinae (Tate 1945). Bensley (1903) regarded *Dromicia* (= *Cercaërtus*) as one of the most primitive Phalangerinae showing an approximation to a (hypothetical) insectivorous prototype. He considered this prototype to have the dental characteristics of the more primitive Peramelidae and hence labelled it Properamelidae. Bensley considered that *Petaurus* and *Trichosurus* occupied derived positions, in two separate lines, with reference to *Cercaërtus*. *P. breviceps* has 22 chromosomes which are mostly metacentrics (Drummond 1933) and *T. vulpecula* has 20 chromosomes which are nearly all acrocentric (Koller 1936). If the chromosome number and morphology, as found in two species of *Cercaërtus*, does represent the primitive condition for the Phalangerinae then chromosomal evolution in this group has presumably been towards higher numbers which is at variance with the supposition above, that evolution in marsupials has been accompanied by reduction of chromosome numbers. However, White (1957*a*) believes that evolutionary increases in chromosome number by "fragmentations" are unlikely to have occurred and Bensley may have interpreted convergent features of the dentition of *Cercaërtus* as evidence of primitive status. *C. concinnus*, alone amongst Phalangeridae so far studied, has delayed implantation which is probably of general occurrence in the more advanced family Macropodidae (Sharman 1959).

The family Macropodidae is very well known cytologically. In the larger of its two subfamilies (Macropodinae) representatives of all genera except *Dendrolagus*, *Dorcopsis*, and *Dorcopsulus* have been studied. Most species have 22 or 16 chromosomes.

Thylogale stigmatica and *T. thetis* have 22 chromosomes alike in morphology in the two species. One pair of the smaller autosomes are metacentric in both species whereas McIntosh and Sharman (1953) considered that none of the smaller autosomes of *T. billardierii* were metacentric. It is difficult to see the centromere constriction in the smaller autosomes in all three species and a re-examination of

T. billardierii indicates that it has in fact a small pair of metacentric autosomes. The chromosomes of the three species are therefore very similar and in each species the *X*-chromosome has a pronounced undercondensed segment near the centromere. There thus appears to be no justification, on cytological grounds, for Ride's (1957) removal of *T. billardierii* to the genus *Protemnodon*, seven species of which have 16 or, in one case, less than 16 chromosomes. Chromosome counts of *T. stigmatica* and *T. thetis* had not been made when Ride's (1957) paper was written.

Petrogale penicillata and *P. rothschildi* also have 22 chromosomes which are like those of the three species of *Thylogale* in every detail. The possibility cannot be overlooked that the resemblances are due to parallel development of the same chromosome number and morphology in two genera not closely related. This appears unlikely since in three other genera of macropod marsupials with 22 chromosomes (*Setonix, Bettongia,* and *Hypsiprymnodon*) three unlike chromosome morphologies, all different from that of *Thylogale* and *Petrogale*, occur. Two of these, compared with that of one species of *Petrogale*, are shown in Figure 6. The chromosome number and morphology in *Lagorchestes hirsutus* is also very similar to that of *Thylogale* and *Petrogale* and it is considered that the three genera form a natural group. *Lagostrophus fasciatus*, with 24 chromosomes, differs from *Lagorchestes* (with which it was originally included), and from all other members of the subfamily Macropodinae, in the morphology of its urogenital system (Ride, Sharman, and Tyndale-Biscoe, unpublished data) and its placement in a separate genus as proposed by Thomas (1887) is justified.

It was earlier shown (Sharman 1954) that the chromosome number of *Protemnodon eugenii* supports the conclusions of Raven and Gregory (1946) and Tate (1948) who, on anatomical evidence, removed this species from *Thylogale*. The six species of *Protemnodon* with 16 chromosomes (Table 1) appear to constitute a natural group showing a pronounced cytological resemblance to *Macropus major* and some resemblance to *M. robustus*. The latter species, although having the same chromosome number, differs slightly in chromosome morphology. The grouping of the three kangaroos, *M. major, M. robustus,* and *M. rufus* together is largely a matter of convenience and they probably have little in common, except similar size, to justify their inclusion in a single genus. The danger of using size as a criterion of relationship in macropods was stressed by Ride (1957) who believed that the use of size as a primary key character had obscured the true relationships of *P. parma* since 1888. *M. major* and the wallabies included in *Protemnodon* (except *P. bicolor*) have the same chromosome number and similar chromosome morphology and may well be congeneric. If this is so the name *Protemnodon*, which Troughton (1959) believed invalid, should be replaced by the earlier *Macropus* which would embrace *M. major* and all *Protemnodon* listed in Table 1 except possibly *P. bicolor*.

No constant detectable differences were found between the chromosomes of the various forms of *M. major* from different regions (Table 1). These are here regarded as subspecies, as are *ocydromus* and *tasmaniensis* previously studied by McIntosh and Sharman (1953), but are sometimes given specific rank. There is

no cytological evidence indicating that several distinct species are present but this possibility is not precluded by this study. Binder's (1927) count of 22 chromosomes for *M. giganteus* (= *M. major*) is incorrect.

P. bicolor is cytologically quite unlike any other species of the subfamily Macropodinae. Its chromosome number (11♂, 10♀) is very low — perhaps the lowest in any vertebrate animal. The mechanism by which the neo sex-chromosome system arose is clear but this does not help in determining the relationships of the species since no other species of *Protemnodon* (nor any other marsupial) has a chromosome complement which could be regarded as the precursor of the condition found in *P. bicolor*. As Ride (1957) suggested the genus *Protemnodon* may be polyphyletic (or at least diphyletic) and the anatomical resemblances of *P. bicolor* to other species of the genus may be due to parallelism. It is thus possible that *bicolor* should be removed from *Protemnodon* and placed in a monotypic genus, *Wallabia* Trouessart, 1905, of which it is the genotype since, in other genera of marsupials, all species so far studied have the same chromosome number and morphology (*Macropus* is an exception but this genus, as constituted in Table 1, is probably also polyphyletic). However, in some genera of insects with otherwise constant chromosome numbers one or more species may be very different cytologically (White 1957*b*) and many genera of eutherian mammals have a considerable range of chromosome numbers (Matthey 1958).

It was earlier shown (McIntosh and Sharman 1953) that the number of chromosome arms (what Matthey (1949) calls the "nombre fondamental") is reasonably uniform in a series of macropod marsupials with different chromosome numbers. Thus *M. rufus*, although differing in chromosome number from *M. major* and *M. robustus*, has about the same number of chromosome arms (Table 2). This may be an indication that the 16 chromosomes of *M. major* and *M. robustus* are derived from a larger number by the occurrence of several centric fusions but, in any event, the difference in chromosome number is probably sufficient to justify the placing of *M. rufus* in the separate genus *Megaleia* as is sometimes done. There are several objections to assuming that Robertsonian changes (i.e. lowering of chromosome numbers by centric fusions between non-homologous chromosomes) have been important in chromosomal evolution in marsupials. The first of these is the difficulty of defining exactly what constitutes a chromosome arm especially in species such as *Petrogale rothschildi* (Fig. 3) and *M. robustus* (Fig. 4) where it is uncertain whether one or more pairs of rod-shaped chromosomes with terminal "knobs" should be defined as one-armed or two-armed. This difficulty is not encountered in the insectivore *Sorex araneus*, which is polymorphic for three centric fusions (Sharman 1956; Ford, Hamerton, and Sharman 1957), since in this species each chromosome can at once be designated acrocentric or metacentric. This statement does not of course imply that there is no short arm in the acrocentric chromosomes of *Sorex*. A further objection is that the number of chromosome arms present does not give any indication of the number of tandem fusions that have taken place. Thus a metacentric chromosome may be formed by the union of two acrocentrics (as are the smaller metacentric chromosomes of some individuals of *S. araneus*) or it may be formed by the union of one acrocentric

and one metacentric chromosome, as are the compound *X*-chromosomes of *Sorex* and two species of marsupials. To put this another way: the "nombre fondamental" of *Protemnodon bicolor* is 20 (Table 2) but its (hypothetical) ancestor had 22 chromosome arms. Perhaps *P. bicolor* and *Potorous tridactylus* have many less chromosome arms than related species (Table 2) because a number of tandem fusions have occurred in their ancestry.

Onychogalea unguifer also has less than the usual number of chromosome arms (Table 2). In this species recent incorporation of autosomal material into the sex chromosomes may account for their large size and unusual behaviour at

TABLE 2

CHROMOSOME NUMBER AND "NOMBRE FONDAMENTAL" IN SOME MACROPOD MARSUPIALS

Species	Chromosome Number	Nombre Fondamental (in females)
Macropodinae		
Protemnodon bicolor	11♂, 10♀	20
Protemnodon (5 spp.), *Macropus major, M. robustus*	16	28–30
Macropus rufus	20	28–30
Onychogalea unguifer	20	26
Setonix brachyurus	22	36–40
Potoroinae		
Potorous tridactylus	13♂, 12♀	24
Hypsiprymnodon moschatus	22	28
Bettongia (3 spp.)	22	40

mitosis and meiosis. If this has happened *O. unguifer* may be derived from a form with 22 chromosomes, like *Petrogale, Thylogale,* or *Lagorchestes hirsutus.* We earlier suggested (Sharman and Barber 1952) that the multiple sex-chromosome systems of marsupials may represent a stage in the evolution of a new *XY* system and it is possible that in *O. unguifer* the Y_1-chromosome of a multiple system has disappeared thus producing a new *XY* system.

Setonix brachyurus has more than the usual number of chromosome arms (Table 2). It was earlier stated that the chromosome number of this species may indicate relationship to *Thylogale,* which also has 22 chromosomes, but chromosome morphology is very different in the two genera. Several of the larger autosomes of *Setonix* are definitely metacentric and nearly all of the smaller autosomes have a more or less minute short arm (Sharman 1954). The chromosome number and morphology in *Setonix* is rather like that found in the rat-kangaroo *Bettongia* (Fig. 6) as Ride and Sharman (in Ride 1957) pointed out but it is not implied that *Setonix* is necessarily a rat-kangaroo. Wood Jones (1924) grouped *Setonix* with *Dendrolagus* and *Dorcopsis* which have not yet been studied cytologically.

The subfamily Potoroinae (rat-kangaroo), although having fewer living species than the Macropodinae, is the more diversified of the two groups (Tate 1948). The three genera thus far studied cytologically have very different chromosomes. Those of *Hypsiprymnodon*, which, in some respects, is one of the most primitive of the kangaroo-like marsupials, are more like those of genera such as *Thylogale* and *Petrogale*, than they are like those of *Bettongia* or *Potorous*, which are themselves very different cytologically. Two monotypic genera of rat-kangaroos (*Aepyprymnus* and *Caloprymnus*) have not yet had their chromosomes studied and it is not possible, at present, to comment on relationships within the group.

V. Acknowledgments

Cytological material, fixed in the field or laboratory, was presented by Professor Ida Mann, Drs. G. M. Dunnet, S. J. Edmonds, J. L. Harrison, W. D. L. Ride, Mrs. Jennifer M. Barker, Messrs. J. H. Calaby, E. H. M. Ealey, C. H. Tyndale-Biscoe, and J. McNally. Miss Phyllis E. Pilton, Messrs. S. Barker, C. D. Gooding, B. J. Marlow, and A. E. Newsome assisted with collecting some of the species in the field. The help of all of these is gratefully acknowledged.

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THE HAEMATOZOA OF AUSTRALIAN REPTILES

By M. Josephine Mackerras*

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Contents

Summary

Trypanosomes are known from tortoises, a gecko, and skinks, *Trypanosoma phylluri* from *Phyllurus platurus,* and *T. egerniae* from *Egernia striolata* and *E. cunninghami* being new. There is an old record of a trypanosome in a snake, but the organism has not been rediscovered.

Haemogregarines are common in all groups. Names had already been given to 2 from tortoises (one of which is regarded as a synonym), 4 from lizards, and 10 from snakes in Australia. Three species described from snakes in Asia have been added, and 15 new species are described from the following type hosts: *Haemogregarina heteronotae* from *Heteronota binoei*; *Hg. palmeri* from *Physignathus lesueurii*; *Hg. taeniolati* from *Sphenomorphus taeniolatus*; *Hg. cunninghami, Hg. egerniae,* and *Hg. obscura* from *Egernia cunninghami*; *Hg. johnstoni* from *Varanus varius varius*; *Hg. breinli* and *Hg. gilruthi* from *Varanus tristis orientalis*; *Hg. stegonoti* from *Stegonotus plumbeus*; *Hg. boigae* from *Boiga fusca*; *Hg. australis* and *Hg. eidsvoldensis* fom *Pseudechis australis*; *Hg. denisoniae* from *Denisonia pallidiceps; Hg. aspidomorphi* from *Aspidomorphus harriettae.*

Two species of *Plasmodium* are known from lizards, *P. egerniae* from *Egernia major major* being new.

The genus *Haemocystidium* is revived, one species being recognized in freshwater tortoises and one in geckos.

Pirhemocyton has been found in two species of geckos and a carpet snake.

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I. Introduction

Dr. E. A. Johnson was the first to bestow a name on a blood parasite of an Australian animal, when he described and named *Trypanosoma chelodina* in a tortoise in 1907. Sambon in England, Johnston and Cleland in Sydney, Gilruth, Sweet, and Dodd in Melbourne, and Lewis in Darwin soon added to our knowledge of the blood parasites of reptiles, but for many years now no work appears to have been done on the subject.

Johnston (1932) recorded ecto- and endoparasites of *Trachysaurus rugosus* (Gray), this account including intestinal flagellates, ciliates, and amoebae, as well as helminths, but no blood parasites.

The reptiles are a very ancient group, and one might expect to find their parasites widely distributed. On the other hand, individuals of some species, particularly lizards, have restricted ranges, and this might lead to the development of isolated races or species of parasites, which could then have a very circumscribed distribution. Careful study of hosts and parasites from many localities is clearly required.

II. Materials and Methods

The material has been collected intermittently over many years, and consists mainly of thin blood films, which were dried in air, and stained by one of the Romanowsky methods. Sections and smears of organs have also been searched when they were available.

The classification of the hosts follows Loveridge (1934) and Kinghorn (1956), with some modifications.

As in previous publications in the series (Mackerras 1959; Mackerras and Mackerras 1960), the following abbreviations have been used in the descriptions of trypanosomes:

- L Total length, measured along the mid-line, and excluding the free flagellum.
- B Maximum breadth, including the undulating membrane, usually measured near the nucleus.
- PK Distance from posterior end to kinetoplast.
- KN Distance from kinetoplast to the posterior edge of the nucleus.
- NA Distance from anterior edge of nucleus to anterior end.
- FF Free flagellum.

III. Host–Parasite List

Classification and Name of Host	Parasite
TESTUDINES	
Chelidae	
Chelodina longicollis (Shaw, 1802), long-necked tortoise	*Trypanosoma chelodina* Johnson
	Haemogregarina clelandi Johnston
	Haemocystidium chelodinae Johnston & Cleland

Classification and Name of Host	Parasite
C. oblonga Gray, 1841, oblong tortoise	*Haemogregarina clelandi* Johnston *Haemocystidium chelodinae* Johnston & Cleland
C. expansa Gray, 1857, lagoon tortoise	*Haemogregarina clelandi* Johnston
Emydura macquarii (Gray, 1831), Murray tortoise *E. latisternum* (Gray, 1867), saw-toothed tortoise *E. krefftii* (Gray, 1871), Krefft's tortoise *Elseya dentata* (Gray, 1863), overlander	*Trypanosoma chelodina* Johnson *Haemogregarina clelandi* Johnston *Haemocystidium chelodinae* Johnston & Cleland
SQUAMATA, suborder LACERTILIA	
Gekkonidae	
Phyllurus platurus (Shaw, 1790), leaf-tailed gecko	*Trypanosoma phylluri*, sp. nov. *Haemogregarina* sp. *Haemocystidium simondi* Castellani & Willey *Pirhemocyton tarentolae* Chatton & Blanc
Heteronota binoei Gray, 1845, Bynoe's gecko	*Haemogregarina heteronotae*, sp. nov. *Haemocystidium simondi* Castellani & Willey
Oedura tryoni de Vis, 1884, Tryon's gecko	*Haemocystidium simondi* Castellani & Willey
Gehyra variegata australis Gray, 1845, variegated gecko	*Haemogregarina* sp. *Haemocystidium simondi* Castellani & Willey *Pirhemocyton tarentolae* Chatton & Blanc
Agamidae	
Amphibolurus barbatus (Cuvier, 1829), jew lizard	*Plasmodium giganteum* Theiler
Physignathus lesueurii (Gray, 1831), eastern water-dragon	*Haemogregarina palmeri*, sp. nov.
Scincidae	
Sphenomorphus quoyii (Duméril & Bibron, 1839), water lizard	*Haemogregarina hinuliae* Johnston & Cleland
S. taeniolatus (Shaw, 1790), copper-tailed skink	*Trypanosoma* sp. *Haemogregarina taeniolati*, sp. nov.
Tiliqua scincoides (Shaw, 1790), blue-tongued lizard	*Haemogregarina tiliquae* Johnston & Cleland
Egernia cunninghami (Gray, 1832), spiny skink	*Trypanosoma egerniae*, sp. nov. *Haemogregarina cunninghami*, sp. nov. *Haemogregarina egerniae*, sp. nov. *Haemogregarina obscura*, sp. nov.
E. major major (Gray, 1845), land mullet	*Plasmodium egerniae*, sp. nov.
E. striolata (Peters, 1870), arboreal skink	*Trypanosoma egerniae*, sp. nov. *Haemogregarina cunninghami*, sp. nov. *Haemogregarina egerniae*, sp. nov.
Varanidae	
Varanus varius varius (Shaw, 1790), tree goanna	*Haemogregarina varanicola* Johnston & Cleland *Haemogregarina johnstoni*, sp. nov. *Haemogregarina breinli*, sp. nov.

Classification and Name of Host	Parasite
V. varius belli (Duméril & Bibron, 1836), Bell's goanna	*Haemogregarina varanicola* Johnston & Cleland
V. tristis orientalis Fry, 1913	*Haemogregarina gilruthi*, sp. nov. *Haemogregarina breinli*, sp. nov. *Haemogregarina varanicola* Johnston & Cleland
V. gouldii (Gray, 1838), sand goanna	*Haemogregarina varanicola* Johnston & Cleland *Haemogregarina gouldii* Johnston & Cleland *Haemogregarina johnstoni*, sp. nov. *Haemogregarina breinli*, sp. nov.
Suborder OPHIDIA	
Boidae	
Liasis fuscus Peters, 1873, brown rock-python	*Haemogregarina fuscus* Lewis
L. amethystinus kinghorni Stull, 1933, North Queensland python	*Haemogregarina pythonis* (Billet) *Haemogregarina shattocki* Sambon & Seligmann *Haemogregarina amethystina* Johnston
Morelia spilotes spilotes (Lacépède, 1804), diamond snake	*Haemogregarina pythonis* (Billet) *Haemogregarina shattocki* Sambon & Seligmann
M. spilotes variegata Gray, 1842, carpet snake	*Haemogregarina pythonis* (Billet) *Haemogregarina pococki* Sambon & Seligmann *Haemogregarina shattocki* Sambon & Seligmann *Haemogregarina amethystina* Johnston *Haemogregarina moreliae* Johnston *Haemogregarina megalocystis* Gilruth, Sweet, & Dodd *Pirhemocyton* sp.
Chondropython viridis (Schlegel, 1872), green tree-python	*Haemogregarina pythonis* (Billet)
Colubridae	
Dendrophis punctulatus (Gray, 1827), green tree-snake	*Haemogregarina dendrophidis* Johnston & Cleland *Haemogregarina boigae*, sp. nov.
D. calligaster Günther, 1867, northern green tree-snake	*Haemogregarina calligaster* Lewis
Natrix mairii (Gray, 1841), fresh-water snake	*Haemogregarina mirabilis* Castellani & Willey
Stegonotus plumbeus (Macleay, 1884), Herbert River snake	*Haemogregarina stegonoti*, sp. nov. *Haemogregarina ?aspidomorphi*, sp. nov.
Boiga fusca (Gray, 1842), night-tiger	*Haemogregarina boigae*, sp. nov. *Haemogregarina ?mirabilis* Castellani & Willey *Haemogregarina* sp.
Elapidae	
Pseudechis porphyriacus (Shaw, 1794), red-bellied black snake	*Haemogregarina pseudechis* Johnston *Haemogregarina denisoniae*, sp. nov.
P. australis (Gray, 1842), mulga snake	*Haemogregarina pseudechis* Johnston *Haemogregarina bancrofti* Johnston & Cleland *Haemogregarina australis*, sp. nov. *Haemogregarina eidsvoldensis*, sp. nov. *Haemogregarina darwiniensis* Lewis

Classification and Name of Host	Parasite
P. guttatus de Vis, 1905, spotted black snake	*Haemogregarina bancrofti* Johnston & Cleland *Haemogregarina ?shattocki* Sambon & Seligmann
Denisonia pallidiceps (Günther, 1858), little black snake	*Haemogregarina denisoniae*, sp. nov.
D. signata (Jan, 1859), marsh snake	*Haemogregarina denisoniae*, sp. nov.
Aspidomorphus harriettae (Krefft, 1869), white-crowned snake	*Haemogregarina aspidomorphi*, sp. nov.
Notechis scutatus (Peters, 1861), tiger snake	*Haemogregarina aspidomorphi*, sp. nov. *Haemogregarina denisoniae*, sp. nov. *Haemogregarina bancrofti* Johnston & Cleland *Haemogregarina ?shattocki* Sambon & Seligmann
Acanthophis antarcticus (Shaw, 1794), death adder	*Haemogregarina* sp.
Demansia psammophis reticulata (Gray, 1842), spinifex snake	*Haemogregarina* sp.
?*D. textilis* (Duméril & Bibron, 1854), brown snake	*Trypanosoma* sp.

IV. Class MASTIGOPHORA

Genus TRYPANOSOMA Gruby

Trypanosoma chelodina Johnson

Trypanosoma chelodina Johnson, 1907, p. 26.

Hosts.—*Chelodina longicollis* (Shaw) (type host), *Emydura krefftii* (Gray), *E. macquarii* (Gray), *E. latisternum* (Gray), *Elseya dentata* (Gray).

Distribution.—Morgan, S. Aust. (type locality); Sydney, N.S.W.; Brisbane, Mt. Nebo, Eidsvold, Townsville, Qld.

This species was originally described from *C. longicollis* from Morgan. Johnson's brief account was reprinted by Johnston (1909*b*), and corrected and amplified by Johnston and Cleland (1912). Measurements and figures were given for trypanosomes from *E. macquarii* from Queensland, as well as for those present in a slide prepared by Dr. E. A. Johnson, presumably from the type host. Breinl (1913) also found this parasite in 2 out of 10 *C. longicollis* examined in Townsville.

Johnston and Cleland described the parasites as possessing a fairly constant form. The anterior end was long and narrow, ending acutely; the main mass of the body was of fairly uniform breadth, tapering rapidly at the posterior end; and the cytoplasm was usually homogeneous, though a few large vacuoles were seen in some specimens. The kinetoplast lay at about one-third of the distance between the end and the centre, and the large rounded nucleus lay slightly posterior to the centre. The undulating membrane was rather wide. The authors gave the following dimensions: L, 39·5–43 μ; B, 5–8·5 μ; PK, 5·8 μ; KN, 10·12 μ; NA, 18·5–21·5 μ; FF, 3–6 μ.

Infections have been studied in *C. longicollis* from Brisbane, *E. latisternum* from Mt. Nebo, *E. krefftii* and *Elseya dentata* from Eidsvold.

Morphology (Plate 1, Figs. 1 and 2)

In the living state, the trypanosomes were observed to wriggle to and fro, and rotate actively, with rapid movements of the undulating membrane and flagellum. They did not, however, make much progress among the blood cells. In stained films, the parasites were uniform in size, and no dividing forms were seen. They agreed with the account given by Johnston and Cleland, with some exceptions: they were slightly shorter, the nucleus was usually near the centre, but not always definitely posterior to it, and the flagellum was much longer. Some of the trypanosomes appeared to have a short flagellum, but this only occurred where the organism was crowded among red cells, and it was thought that the terminal portion of the flagellum might be obscured. Wherever an organism was found lying clear of blood cells in a well-stained film, the flagellum was at least 15 μ long (Figs. 1 and 2). A long free flagellum was clearly shown also in a photomicrograph sent by the Rev. R. Palmer of a trypanosome in the blood of *Chelodina longicollis* taken near Sydney.

Dimensions of forms from E. krefftii.—L, 34–38 μ; B, 4·5–8 μ; PK, 5–9 μ; KN, 8–16 μ; NA, 11–17 μ; FF, 15–19 μ.

Relationships and Life History

This species is evidently close to *T. vittatae* Robertson, 1908, from a Ceylon tortoise, but differs from it in being smaller, and in the nearly central position of the nucleus, which is definitely in the posterior half of the body in *T. vittatae*. Robertson (1909) showed that *T. vittatae* was transmitted by an aquatic leech, *Glossiphonia* sp. It is probable that *T. chelodina* has a similar life history, but efforts to induce several small aquatic leeches to bite a long-necked tortoise were unsuccessful.

TRYPANOSOMA PHYLLURI, sp. nov.

Host.—Phyllurus platurus (Shaw).

Distribution.—Mosman, French's Forest, Narrabeen, all in the Sydney district, N.S.W.

Type.—Slide from *P. platurus* from French's Forest, near Sydney, in the Queensland Museum, Brisbane.

Infections were found in 11 out of 69 leaf-tailed geckos examined from various localities on the Hawkesbury sandstone north of Sydney.

Morphology (Plate 1, Figs. 3 and 4)

An extremely flattened, delicate organism, the cytoplasm being very thin, fragile, and easily torn or distorted. Most of the infections were scanty. The parasites were fairly uniform in size, and no dividing forms were seen. When alive, the organisms often rotated in the one spot, and waves were seen to run in either direction along the undulating membrane. In stained films, the cytoplasm appeared pale blue or grey, with fine granules. The kinetoplast lay close to the nucleus, and usually stained reddish. The posterior end was pointed, the anterior

end was sometimes difficult to define, and the flagellum was very short. The nucleus was small, 2 by 1 μ, compact, oval or bean-shaped, and situated about the mid-point of the body.

Dimensions.—L, 36–48 μ; B, 7–15 μ (usually 14 μ); PK, 14–21 μ; KN, 0–1 μ; NA, 20–25 μ; FF, 2–6 μ.

Experimental

Citrated heart blood from a gecko with a scanty infection was injected intraperitoneally into three others, in which trypanosomes had not been seen. The blood of the recipients was examined weekly for 3 weeks, and again after 3 months, but no trypanosomes were seen.

Relationships

This species seems to be most closely related to *T. pertenue* Robertson from the geckos, *Hemidactylus triedri* and *H. leschenaultii*, from Ceylon. *T. pertenue* has a small compact nucleus lying close to the kinetoplast near the middle of the body, a disposition very similar to that seen in *T. phylluri*. However, the body is narrower and the free flagellum considerably longer than in *T. phylluri*.

It is also close to *T. platydactyli* Catouillard from *Platydactylus muralis* from Tunis. This species is, however, narrower than *T. phylluri*; the nucleus and kinetoplast lie close together but are nearer the posterior end of the body; the undulating membrane has more undulations; and the free flagellum is much longer (Catouillard 1909).

T. leschenaultii Robertson from *Hemidactylus leschenaultii* is a very distinctive species. It is a long organism, of moderate breadth, with a well-developed undulating membrane with numerous undulations, and a long free flagellum. The kinetoplast lies a considerable distance from the posterior end, but is well separated from the large oval nucleus.

TRYPANOSOMA EGERNIAE, sp. nov.

Hosts.—*Egernia striolata* (Peters), *E. cunninghami* (Gray).

Distribution.—Sydney; Eidsvold, Qld.

Type.—Slide from *E. striolata* from Eidsvold, in the Queensland Museum, Brisbane.

Morphology (Plate 1, Figs. 5–7)

The parasites were fairly numerous in films from 1 out of 7 *E. striolata* from Eidsvold. They were nearly uniform in size, and presented a characteristic appearance in stained films, the undulating membrane being thrown into 3 or 4 large undulations, and usually folded back across the body, giving it a peculiarly angular appearance. The organism appeared to be an elongate, flattened blade, usually with a spiral twist. When seen from the flattened side the ends appeared rounded (Fig. 5), but when seen in profile the ends appeared pointed (Fig. 6). The cytoplasm was thin, but dense, granular, and deeply stained. The kinetoplast was very small, and usually placed near a large elongate vacuole. The flagellum

was very delicate and usually difficult to make out. The nucleus could not be made out; it was presumably obscured by the deeply staining cytoplasm. The breadth given below was measured at the level of the kinetoplast.

Dimensions.—L, 22–28 μ; B, 4–5 μ; PK, 5–8 μ; FF, 11–12 μ.

A single trypanosome was found in a slide from 1 out of 9 *E. cunninghami* from Sydney (Fig. 7). It was larger than any of the type series, but resembled them closely in other respects.

Trypanosoma sp.

Trypanosomes were recorded by Johnston and Cleland (1912) as being present in a slide from *Sphenomorphus taeniolatus* (Shaw), sent to them from Eidsvold by Dr. T. L. Bancroft. They did not describe or name it. Scanty trypanosomes have been seen in 1 out of 30 *S. taeniolatus* from French's Forest and other places north of Sydney. Apparently it is not an abundant parasite in these skinks. No material suitable for description is as yet available.

Trypanosoma sp.

Johnston and Cleland (1910) recorded the occurrence of trypanosomes in a "brown snake", which they provisionally placed as *Diemenia textilis* Duméril & Bibron. The record was based on the account of a meeting of the Queensland Branch of the British Medical Association on July 13, 1906, at which Dr. Wilton Love exhibited a trypanosome in the blood of a brown snake sent by Dr. Baxter Tyrie, of Boonah, in southern Queensland. This organism has not been reported again.

V. Class SPOROZOA

Order COCCIDIA

Classification of Haemogregarines

The haemogregarines of reptiles have been described as belonging to several genera distributed in two suborders, namely: *Haemogregarina, Hepatozoon,* and *Karyolysus* in Adeleidea, and *Schellackia* in Eimeriidea.

In *Haemogregarina* Danilewsky, 1885, s.s., schizogony occurs in the red blood cells and organs of the vertebrate host, and sporogony occurs in leeches. Trophozoites, schizonts, and gametocytes are found in the red cells. The type of the genus is *H. stepanowi* Danilewsky of the European tortoise, *Emys orbicularis,* and the leech *Placobdella catenigera.* The sporozoites are introduced into the tissues of the tortoise by the bite of the leech.

In *Hepatozoon* Miller, 1908, schizogony occurs in the organs of the vertebrate host. The forms in the red or white blood corpuscles are gametocytes. Sporogony occurs in the arthropod host — mite, tick, or blood-sucking insect — and is characterized by the production of very large oocysts, each containing numerous sporoblasts, which in turn contain numerous sporozoites. Infection occurs when the vertebrate eats the arthropod. The type of the genus is *Hepatozoon muris* (Balfour, 1906) of *Rattus* spp. and the mite, *Echinolaelaps echidninus.*

Species of *Hepatozoon* have been described from mammals and birds, and the studies of Hoare (1932) on the transmission of *H. pettiti* of the crocodile by certain tsetse flies (which habitually feed in the crocodile's mouth, and hence get eaten occasionally) and of Robin (1936) on the transmission of *H. mesnili* of a gecko by mosquitoes, showed that some haemogregarines of reptiles are also referable to this genus. Garnham (1950) described *Hepatozoon minchini* from a swamp snake in Kenya, placing it in this genus from the appearance of the schizonts in the lungs.

In *Karyolysus* Labbé, 1894, schizogony occurs in the endothelial cells of capillaries in various organs of the vertebrate host. The gametocytes are found in the red blood cells. Sporogony occurs in mites, and vermiform sporoblasts enter the ova and complete their development in the larval and nymphal mites of the next generation. Infection of the vertebrate takes place when it eats the mite, or cysts voided in the mite's faeces. The type of this genus is *Karyolysus lacertarum* (Danilewsky, 1886) of the European lizard, *Lacerta muralis*, and the mite, *Liponyssus saurarum*. Reichenow (1913) studied this parasite and elucidated the life history given above. He found two kinds of schizonts in the liver of the lizard, one producing 8–30 macromerozoites, which entered other endothelial cells, and the other producing numerous micromerozoites which entered red cells and developed into gametocytes.

Labbé proposed the name *Karyolysus* because the parasite had a destructive effect upon the nucleus of the host cell, causing it to become swollen and elongated, but Wenyon (1926) did not regard the karyolytic action as of generic value.

In *Schellackia* Reichenow, 1920, the whole development is said to take place in the vertebrate host. Schizogony and sporogony were described in the epithelium of the gut. The male gametocyte produced a comparatively large number of microgametes which fertilized the macrogametes. The oocyst developed in the subepithelial layers of the intestine, liberating eight sporozoites which reached the blood stream and entered red cells. They were taken up by mites, which acted as transmitting agents, but in which no further development occurred, except for growth. The vertebrate was infected by eating the mite (Reichenow 1920, 1953). No proven members of this genus are known so far from Australian reptiles. It is related to *Lankesterella* Labbé, which occurs in birds and frogs.

It is evident that the haemogregarines of reptiles cannot be correctly placed until life history studies have been made. Probably our species from tortoises is a true *Haemogregarina*, and most of those from lizards and snakes belong to *Karyolysus*; but there is no certainty, and they will all be referred to *Haemogregarina* (s.l.) in this paper.

Observations on Australian Species

Two clearly defined groups occur in lizards and snakes in Australia, as in other parts of the world. In one group, the infected red cell becomes greatly enlarged, far in excess of the requirements of the parasite for space. The cell envelope becomes stretched, the cytoplasm stains abnormally, and the nucleus becomes distorted, sometimes swollen, and even fragmented. The actual parasite

is usually a rather small sausage-shaped body, which is closely applied to the nucleus of the host cell. Two members of this group have already been described in Australia: *Hg. bancrofti* from *Pseudechis* spp., and *Hg. megalocystis* from *Morelia spilotes variegata*. Two others are described in this paper.

The second group contains the majority of known forms. The infected cells are only slightly, if at all, enlarged. Any enlargement is mainly in the long axis, and appears to be due to the mechanical effect of a rather big parasite stretching the cell envelope, and not to any toxic action, as in the first group. The cell cytoplasm stains normally, and its nucleus is not swollen but may be displaced.

Some species are intermediate in their effect on the host cell, *Hg. gouldii* causing elongation of the red cell nucleus, and cells parasitized by *Hg. boigae* and *Hg. stegonoti* frequently having swollen nuclei.

Immunity

Apparently reptiles do not develop much immunity to these parasites. Young reptiles are seldom heavily infected, but old ones frequently are. It seems that these infections are built up steadily over the years and that a state of tolerance develops. This is in contrast to the condition found in many mammals and birds infected with various blood parasites. In these groups, heavy infections are usually found in young animals, and the duration of parasitaemia is usually short; older animals may show scanty parasites or none at all, and they may be resistant to reinfection. It seems possible that immunological mechanisms may be less developed in the reptiles than in higher groups.

Nothing is known about host specificity in the haemogregarines of reptiles. It has been assumed, provisionally, that members of one family are probably susceptible to the same species of haemogregarines, and that members of related families may also be susceptible. In the Ophidia, however, there are a few puzzling cases, in which quite similar haemogregarines have been found in what appear to be distantly related hosts.

Development in the Vertebrate Host

It seems that there is still a great deal to be learnt about these parasites. In December, 1907, Minchin apologized for making yet another new species of haemogregarine, and added the pertinent observation that what was required was not more new species, but new facts about the old species. In spite of the lapse of more than half a century, this is equally true today.

Reichenow (1910) studied *Hg. stepanowi* in a tortoise, and concluded that the sporozoites introduced by the intermediate host entered red corpuscles. In the light of what is now known of the malaria parasites, in which this was formerly said to occur, this point requires checking.

Wenyon (1909) described and figured two kinds of schizonts in the liver of a lizard, *Mabuia quinquetaeniata*, infected with *Hg. gracilis*. There were large schizonts producing numerous slender micromerozoites and smaller oval schizonts producing relatively few macromerozoites. Slender encapsulated parasites were present in the red cells, but smaller unencapsulated forms, which were sometimes

oval, were also present in red cells. Wenyon considered that the slender micromerozoites from liver schizonts entered the red cells and developed into gametocytes, and that the macromerozoites entered other liver cells. This interpretation may be correct, but it does not explain the unencapsulated oval parasites in the red cells, nor the function of the small schizonts in the liver.

Two kinds of schizonts have also been reported by other workers, for example, by Labbé himself, by Laveran and Pettit (1909*b*) in *Varanus* and *Psammodromus*, by Wolbach (1914) in *Varanus*, and by Shortt (1917, 1922*b*) in an agamid lizard and a gecko. The larger schizonts were found in one of the organs, and the smaller, which were sometimes thought to be in red cells, were usually regarded as producing gametocytes. Da Cunha and Muniz (1927) found small and large schizonts in a frog infected with *Hg. leptodactyli* Lésage. They interpreted the small schizonts as an asexual cycle derived from sporozoites, and the larger as giving rise to numerous merozoites, which penetrated red cells and became gametocytes.

Some authors originally regarded the vermicule as a stage of development of the schizont, postulating the fusion of the two limbs to form an ovoid body (Reichenow 1910). Minchin and Woodcock (1910) gave the name "schizokinete" to this stage. It is, however, more probable that the vermicule is the mature gametocyte, motility being necessary at this stage in an organism producing isogametes.

In the present study, circulating schizonts have been found only in tortoises, but two types of schizonts were present in the liver or lung of varanids and three species of snakes. For the sake of brevity, these have been referred to as "X" and "Y" schizonts. There was no correlation between the presence of these schizonts and the effect of the parasites on infected red cells.

X schizonts are relatively large conspicuous bodies. In their early stages, they have pale foamy cytoplasm and large, angular, dark, granular nuclei, which contrast markedly with those of the host tissue in sections stained with haematoxylin and eosin. They produce fairly numerous small merozoites with large dark nuclei, similar to the micromerozoites of Wenyon.

Y schizonts are usually slightly smaller, and are certainly less conspicuous than the X schizonts. They are often associated with the heavily pigmented macrophages, which are characteristic of reptilian liver. The merozoites are large, with pale cytoplasm and very small, round, dark nuclei, which also contrast markedly with those of the surrounding host cells. Usually from 1 to 7 merozoites are seen. These are similar to the macromerozoites of Wenyon.

Two distinctive forms are usually present in the peripheral blood. They were figured by Laveran and Pettit (1909*b*), and were observed by Johnston and Cleland and by Lewis in their studies of haemogregarines of reptiles in Australia years ago. Most frequently seen are elongate parasites, which may be slender or broad, but which have a definite capsule limiting the apparent length of the parasite. In some species, the capsule merely bends the ends of the parasite slightly inwards; in the larger forms, the narrower end is acutely flexed against the rest of the parasite, forming the so-called tail. The nucleus is usually of

medium to small size, and is frequently placed nearer the narrower end. The cytoplasm is usually homogeneous, but sometimes contains chromatinic granules. When these forms are mature, they are readily liberated as motile vermicules when the blood is drawn. They then appear in their full length, and progress with the broader end in front. The vermicule is considered to be the mature gametocyte.

The nature of the capsule which surrounds the vermicule is unknown, but it is a very definite structure. It is thick in some species, and prevents the organism from becoming stained. When the vermicule is liberated from the red cell, the capsule sometimes remains in the cell, but usually it is freed by the bursting of the cell, and, in films in which free vermicules are numerous, their empty capsules may be seen as pinkish bodies with straight sides and rounded ends (Plate 4, Fig. 4). Sometimes they split and roll up into two narrow bundles, the nature of which would be puzzling if intermediate stages were not present.

The second form of parasite is relatively short and broad, varying from oval to sausage-shaped, with a large centrally placed nucleus and vacuolated cytoplasm. There is no evidence that a capsule is present. This form is considered to be a developing trophozoite.

A third form, which is only occasionally seen, is a slender crescentic body, with fairly large centrally placed nucleus. It is thought to be a merozoite from an X schizont, and the forerunner of the second form described above.

Hitherto, many observers have postulated that the micromerozoites become gametocytes, and that the macromerozoites enter other fixed cells. It is, however, possible that their roles may be reversed, and, in an attempt to explain the diverse forms seen in the red cells and internal organs, the hypothesis is put forward that, in one group of haemogregarines, the sporozoites from the arthropod host enter endothelial cells, or other fixed cells, in liver, lung, or other organ, and develop into X schizonts. The merozoites produced by them may enter other endothelial cells and repeat the cycle, but eventually some enter red cells, and develop into unencapsulated, ovoid or sausage-shaped parasites with bulky nuclei and vacuolated cytoplasm. When full-grown, these parasites lodge in the internal organs and may be taken up by macrophages. Schizogony takes place, resulting in the formation of Y schizonts, with few large merozoites with small nuclei and uniform cytoplasm. These enter red cells and develop into encapsulated vermicules with small- or medium-sized nuclei and non-vacuolated cytoplasm. They are gametocytes and develop further only when taken up by the intermediate host.

Theoretically, three stages of infection with haemogregarines might be observed in reptiles, if this hypothesis is correct:

Stage 1.—After the introduction of sporozoites and their development into X schizonts in the liver or other organ, the first parasites to appear in the peripheral blood should be unencapsulated forms with large nuclei destined to develop into Y schizonts.

Stage 2.—After the development of Y schizonts and the entry of the large merozoites derived from them into red cells, two kinds of parasites, unencapsulated forms with large nuclei and encapsulated forms with relatively small nuclei, should be found in the peripheral blood, and X and Y schizonts in the organs.

Stage 3.—In the absence of reinfection, X schizonts should gradually disappear from the organs, and only gametocytes should be present in the blood.

It must be admitted that many infections were seen which would not fit into the hypothetical stages mentioned above, and that other possibilities exist. The so-called Y schizonts may belong to a sporogonic cycle, as they bear a resemblance to oocysts. Reichenow found that *Schellackia bolivari* produced octozoic oocysts in the subepithelial tissue of the gut, and they may conceivably occur in other organs. However, it seems unlikely that so many reptiles would be found harbouring mixed infections with a *Karyolysus*, represented by erythrocytic vermicules and X schizonts, and a *Schellackia* represented by unencapsulated forms in the red cells and oocysts in the organs. It seems less unreasonable to assume that, in some cases at least, all the forms seen belong to the one species. If they should eventually prove to belong to *Schellackia*, the unencapsulated forms must be gametocytes and the encapsulated vermicules sporozoites, and, incidentally, one of the distinctions between *Schellackia* and *Lankesterella* would disappear.

Hg. heteronotae, sp. nov., differs from other species in several respects. Thus, although two distinct forms were present in the peripheral blood, and intracellular and free vermicles were numerous, a definite capsule could not be made out around any parasite. X schizonts were abundant in the lung but no Y schizonts were found. On the other hand, small cystic bodies containing single parasites were present in the lung.

The fully developed intracellular vermicule seems to be a relatively stable stage in the life history. It seems reasonable, therefore, to accept morphological differences between mature vermicules, and their effects on the parasitized cells, as good specific characters. If this is correct, then the variety of species encountered was surprising, and mixed infections were common.

The present state of our knowledge of Australian species is summarized in Table 1, in which the blank spaces show where information is still lacking. It is to be remembered, too, that many of the negative records are based on very slender evidence. When the life histories are elucidated, some species with one form in the blood and one kind of schizont in the organs may prove to belong to *Hepatozoon*. If those with two forms in the blood and two kinds of schizonts in the organs prove to belong to *Karyolysus*, the definition of this genus will need amending, as it is usually stated that only gametocytes occur in the red cells.

Haemogregarines of Tortoises

Numerous specific names have been used for the haemogregarines of tortoises in various parts of the world, no less than 19 being listed by Wenyon (1926). The species in Australian tortoises appears to be very similar morphologically to *Hg. nicoriae* of Ceylon tortoises, and schizonts occur in the red cells of both. Robertson (1910) showed that *Hg. nicoriae* belongs to the genus *Haemogregarina* (s.s.), and *Hg. clelandi* is probably congeneric with it.

TABLE 1

HAEMOGREGARINES OF AUSTRALIAN REPTILES—SUMMARY OF AVAILABLE INFORMATION

Species	Typical Hosts	Red Cell		Parasites in Red Cells			Parasites in Liver or Lung	
		Enlargement	Damage to Nucleus	Vermicules	Trophozoites	Schizonts	X Schizonts	Y Schizonts
clelandi Johnston	Chelidae	+	−	+	+	+	−*	−
heteronotae, sp. nov.	Gekkonidae	+	−	+	+	−	+	−
palmeri, sp. nov.	Agamidae	+	−	+	+	−		
tiliquae Johnston & Cleland	Scincidae	+	±	+	+	−		
hinuliae Johnston & Cleland	,,	++	±	+	−	−		
taeniolati, sp. nov.	,,	+	±	+	+	−		
cunninghami, sp. nov.	,,	+	−	+	+	−		
egerniae, sp. nov.	,,	±	−	+	+	−		
obscura, sp. nov.	,,	+++	++	+	−	−		
varanicola Johnston & Cleland	Varanidae	+	±	+	−	−	} +	+
johnstoni, sp. nov.	,,	+++	++	+	+	−		
gouldii Johnston	,,	+	+	+	−	−		
breinli, sp. nov.	,,	±	±	+	+	−		
gilruthi, sp. nov.	,,	+	±	+	+	−		
pythonis (Billet)	Boidae	+	−	+	−	−	−†	−
pococki Sambon & Seligmann	,,	+	−	+	+	−		
shattocki Sambon & Seligmann	,,	+	±	+	+	−	+	+
amethystina Johnston	,,	+	±	+	−	−		
moreliae Johnston	,,	+	−	+	?+	−		
megalocystis, Gilruth, Sweet, & Dodd	,,	+++	++	+	?−	−		
fuscus Lewis	,,	++	+	+	?−	−	+	

* Recorded by Robertson (1910) for a related species. † Recorded by Prowazek (1912).

TABLE 1 (*Continued*)

Species	Typical Hosts	Red Cell		Parasites in Red Cells			Parasites in Liver or Lung	
		Enlargement	Damage to Nucleus	Vermicules	Trophozoites	Schizonts	X Schizonts	Y Schizonts
dendrophidis Johnston & Cleland	Colubridae							
calligaster Lewis	,,							
mirabilis Castellani & Willey	,,							
stegonoti, sp. nov.	,,							
boigae, sp. nov.	,,							
pseudechis Johnston	Elapidae							
bancrofti Johnston & Cleland	,,							
australis, sp. nov.	,,							
eidsvoldensis, sp. nov.	,,							
darwiniensis Lewis	,,							
denisoniae, sp. nov.	,,							
aspidomorphi, sp. nov.	,,							

Haemogregarina clelandi Johnston

Haemogregarina clelandi Johnston, 1909*b*, p. 407. Type slide from *Chelodina oblonga* from Perth, in the Australian Museum, Sydney.
Haemogregarina dentata Lewis, 1913, p. 136.

Hosts.—*Chelodina oblonga* Gray, *C. longicollis* (Shaw), *C. expansa* Gray, *Emydura krefftii* (Gray), *E. macquarii* (Gray), *E. latisternum* (Gray), and *Elseya dentata* (Gray).

Distribution.—Perth, W. Aust.; Murray R., S. Aust.; Gloucester, N.S.W.; Brisbane, Mt. Nebo, Eidsvold, Townsville, Qld.; near Darwin, N.T.

Johnston (1909*b*) described this species from a heavy infection in films made by Dr. J. B. Cleland. The parasites measured from 11 by 5 μ to 13 by 7 μ. The young forms were not encapsulated, and the cytoplasm was usually vacuolated. The position of the parasite within the red cell varied considerably, being lateral to the nucleus, oblique, or terminal. The full-grown parasite was enclosed in a wide capsule. Enlargement of the infected red cells and terminal displacement of their nuclei were usual.

Lewis (1913) described and figured *Hg. dentata* from *E. dentata*, the common tortoise in the Darwin district. Forms were observed with small rounded nuclei placed towards one end of the parasite. These appear very similar to the encapsulated vermicules described below. Breinl (1913) stated that haemogregarines were frequently found in *C. longicollis* in the Townsville district, some infections being particularly heavy. He noted considerable enlargement of the infected red cells. Plimmer (1915) recorded the presence of haemogregarines in a *C. expansa*, which died in the Zoological Gardens, London. It is probable that they were *Hg. clelandi*.

Morphology (Plate 2, Figs. 1–6)

Blood films from four species of tortoises from Eastern Australia have been studied. Infections were found in 3 out of 4 *C. longicollis*, in 3 out of 4 *Emydura krefftii*, in 1 out of 2 *E. latisternum*, and in a single specimen of *Elseya dentata*.

In one specimen of *C. longicollis*, parasites were fairly abundant, young forms, schizonts, and encapsulated vermicules being present. The smallest parasites were oval or slightly crescentic in appearance, each with a fairly large reticular nucleus and pale cytoplasm. They measured from 9–11 μ by 3–5 μ (Fig. 1). No capsule was detected. Schizonts with 4–6 nuclear masses measured 15–16 μ by 5–8 μ. None was found in which the division into merozoites was complete, but it seems likely that 6 or 8 are formed (Figs. 2 and 3).

Most of the parasites were encapsulated vermicules. The appearance differed according to the position of the parasite. If seen in what might be termed the dorsoventral view, the parasite appeared as a plump sausage-shaped body, 13–15 μ by 4–6·5 μ, with a small granular nucleus situated in the middle, or frequently nearer one end. The granular cytoplasm stained pale blue in most individuals but in some it was almost colourless. Reddish granules were seen in some but they were rare. The nucleus sometimes appeared to be in prophase (Fig. 4). When the parasite was seen in lateral view the true shape could sometimes be clearly

made out, one end of the vermicule being doubled up beside the body (Fig. 5). The capsule seemed to be very delicate and was only clearly seen when the organism was viewed in this position. The organisms measured 20–23 μ in total length along the mid-line, with a broad anterior part up to 6 μ in width, sometimes with a small median projection, and tapering to a slender pointed tail. No free vermicules were found in films from *C. longicollis*, but one was seen in a film from *E. krefftii*. This was considerably smaller, being only 14 μ long (Fig. 6). Films from the other two infected *C. longicollis* contained young forms and encapsulated vermicules. Sections of lung and liver from two *C. longicollis* were searched but no schizonts were found.

Films from *E. krefftii* contained numerous encapsulated forms and scanty small forms, some considerably smaller than those in *C. longicollis*, measuring 7–9 μ by 2–4 μ. Only one schizont was found but it was very similar to those described above. The red cells of *E. krefftii* are slightly smaller than those of *C. longicollis*, and seem to be relatively more enlarged and distorted. Normal red cells of *E. krefftii* measured 16–19 μ by 10–12 μ; infected cells 21–23 μ by 12–14 μ. Normal cells of *C. longicollis* measured 18–24 μ by 10–14 μ; infected cells 19–26 μ by 12–14 μ. The host cell nucleus was usually displaced towards one end or laterally. The infections seen in the other tortoises mentioned were too scanty for useful study.

Life History

As stated above, Reichenow (1910) worked out the life cycle of *Hg. stepanowi* in a European tortoise. Robertson (1910), working independently with another species, *Hg. nicoriae*, in Ceylon, came to much the same conclusions. Large schizonts containing about 70 large merozoites were found in the lung, and small schizonts in the peripheral blood. She described two forms in the blood, bean-shaped and recurved, and considered that the former gave rise to the schizonts in the lung. Development in the gut wall of the leech produced eight sporozoites, but she was unable to find them in the proboscis.

Haemogregarines of Lizards

Family Gekkonidae

Several species of haemogregarines have been described from geckos in other parts of the world. These include:

Haemogregarina platydactyli Billet, 1900, from *Platydactylus* sp. from Algeria
Hg. triedri Robertson, 1908, from *Hemidactylus triedrus* from Ceylon
Hg. leschenaultii Robertson, 1908, from *Hemidactylus leschenaultii* from Ceylon
Hg. procteri Shortt, 1922, from *Phyllodactylus elisae* from Persia
Hg. pavlovskiji Zmeev, 1935, from *Gymnodactylus fedschenkoi* from Tadjikistan, U.S.S.R.
Hepatozoon mesnili Robin, 1936, from *Gecko verticillatus* from Saigon
H. burneti Lavier & Callot, 1938, from *Tarentola mauritanica* from Tunis
Hg. dolichopyrena Zmeev, 1938, from *Gymnodactylus caspius* from Turkmenia, U.S.S.R.

The life history of *H. mesnili* has been worked out by Robin (1936), who showed that schizogony occurred in the lungs, liver, and intestine of the gecko, and sporogony in mosquitoes. Two intracorpuscular forms were present in the peripheral blood: encapsulated vermicules, and large oval forms with vacuolated cytoplasm and a large centrally placed nucleus. The author regarded the vacuolated forms as degenerating gametocytes. Small schizonts containing 2–6 merozoites were present in the organs. Large oocysts, characteristic of the genus *Hepatozoon*, were formed in the body cavity of *Culex fatigans* Wied. Infection was by ingestion.

Shortt (1922*b*) showed that *Hg. procteri* undergoes two types of schizogony in the internal organs, minute merozoites being formed within red cells in the capillaries of the liver, spleen, and lung, and larger merozoites in the endothelial cells of the air spaces in the lung. There was apparently nothing characteristic about the forms in the peripheral blood.

H. burneti is a large stout parasite which deforms the red cells apparently by its sheer size. Schizonts were found in the Kupffer cells of the liver and in the endothelial cells of the lung. These are shown as oval bodies containing a relatively small number of long slender merozoites (Lavier and Callot 1938).

The other species are known only from stages in the peripheral blood. Billet (1900) described *Hg. platydactyli* as possessing two forms, which he regarded as male and female. Laveran, however, commenting on his paper, thought that he was possibly dealing with a mixed infection. Robertson (1908) stated that *Hg. triedri* is characterized by the possession of a double capsule, a loose outer one which tended to stain deeply and a delicate inner one. Two forms were found in the red cells, long slightly recurved forms and broader bean-shaped ones. The young forms had no capsule. Both forms frequently showed an irregular body at one end, staining bright red with Giemsa's stain. *Hg. leschenaultii* possessed two free motile forms, one being slender, with uniform cytoplasm, and the other broader, with granular cytoplasm. Two forms were also present in the corpuscles, a long recurved form and a very large broad form. Schizogony was said to occur in the blood, but details were not given. The accounts of the species described by Zmeev (1935, 1938) are not available for comparison.

Haemogregarines have been seen in three species of geckos in Australia. One of them is distinctive, and is described as new. The others have not been named, as the material is too scanty for adequate study, and it may be possible to identify them with described species when more is known about them.

Haemogregarina heteronotae, sp. nov.

Host.—*Heteronota binoei* Gray.

Distribution.—Mornington I., Qld.

Type.—Slide from *H. binoei* from Mornington I., in the Queensland Museum, Brisbane.

Morphology (Plate 3, Figs. 1–11)

Parasites were present in 3 out of 7 lizards obtained in July, 1959.

Most of the parasites seen were very attenuated vermicules, sometimes curving round the periphery of the red cell and almost completely surrounding its nucleus (Fig. 3), sometimes doubled up in an awkward fashion and pushing the nucleus to one end of the cell (Fig. 4). These parasites measured 23–29 μ in total length by 2·5–3 μ at the broader end. The nucleus was elongate and darkly stained. The cytoplasm of the broader end usually took a pinkish stain. No definite capsule could be made out. Scanty asexual forms were also found in two of the lizards. They were somewhat crescentic, sausage-shaped, or ovoid, ranging from 14 by 3 μ to 20 by 8 μ, with numerous sharply defined vacuoles in the cytoplasm, and with a large granular or reticular nucleus, usually centrally placed (Figs. 1 and 2).

Normal cells varied a good deal in size, measuring 14 22 μ in length by 9–13 μ in breadth. Infected cells measured 17–24 μ by 12–15 μ. Their nuclei were often displaced, but not distorted.

In wet preparations, many vermicules became extracellular, gliding swiftly about among the blood cells, and appearing almost uniform in width for about three-quarters of their length, and then tapering a little at the posterior end (Fig. 5).

Numerous X schizonts were found in sections of the lung. They were studied in serial sections, and found to be ovoid or spherical bodies, which varied a good deal in size. The smallest were about 20 μ long by 18 μ in maximum diameter, containing relatively few peripherally arranged nuclei (Fig. 8). The largest schizonts were about 35–38 μ in diameter and contained numerous nuclei (Figs. 9–11). In the most advanced stages, the centre was filled with small, elongate, sometimes radially arranged merozoites, which could not be counted accurately (Fig. 11). It seemed that more than 50 were formed.

No Y schizonts were found in sections of lung, heart, liver, spleen, pancreas, intestine, or brain. However, small cystic bodies, not seen in any other reptile, were abundant in the lungs (Figs. 6 and 7). The whole cyst was about 25 μ in diameter, and contained what appeared to be a host cell with greyish uniform cytoplasm and one rather flattened nucleus (*h.n.*). This cell had usually shrunk away from the cyst wall, so that it was surrounded by a clear space. Within the cell a single elongate parasite was seen lying in a vacuole (*v.*). When cut in longitudinal section, the organism measured 15-16 μ by 3·5–4·5 μ. The nucleus (*p.n.*) was round, dark, granular, and more or less centrally placed, and was surrounded by a clear halo. The cytoplasm contained reddish granules. In transverse section, the parasite appeared as a round body, surrounded by a clear area. If cut near the mid-point, the nucleus was visible. Although hundreds of these objects were examined, only once were two parasites seen within the one cell. It seems unlikely, then, that they normally divide into two.

Experimental

Eleven *Culex fatigans* were fed on geckos with numerous haemogregarines in the blood. These mosquitoes lived well. They were dissected at intervals from 12 hr to 22 days, but no developmental stages were found in them.

HAEMOGREGARINA sp.

At a meeting of the Linnean Society of New South Wales, Mr. A. J. Bearup exhibited a blood film from a gecko, *Phyllurus platurus*, obtained by the Rev. R. Palmer at Glen Davis, N.S.W. (Bearup 1951). This slide showed haemogregarines and *Pirhemocyton tarentolae*. The photomicrograph shown in Plate 2, Figure 7, was made from a film from the same lizard kindly lent by Mr. Palmer. The parasites were enclosed in tough capsules, which prevented the stain from penetrating into them. The infected cells were not markedly enlarged, nor were their nuclei distorted. We did not see this parasite in any of the 69 specimens of the same species examined near Sydney.

HAEMOGREGARINA sp.

Haemogregarines were present in 1 out of 26 *Gehyra variegata australis* Gray examined at Eidsvold. They were elongate forms, varying from 11 by 2 μ up to 18·5 by 3 μ (Plate 2, Fig. 8). They were enclosed in delicate capsules. A free vermicule measured 18 by 2 μ, and had uniform cytoplasm (Plate 2, Fig. 9). Normal red cells measured 16–19 μ by 9–11 μ, with an average of 17 μ by 10 μ; infected cells 17–23 μ by 9–12·5 μ, with an average of 20·5 μ by 11 μ. The host cell nuclei were not affected, except to be displaced sometimes from their normal position.

Neither of these two species can be identified on the information available.

Family Agamidae

HAEMOGREGARINA PALMERI, sp. nov.

Host.—*Physignathus lesueurii* (Gray).

Distribution.—Cobark, near Gloucester, N.S.W.

Type.—Slide from *P. lesueurii* from Cobark, collected by Rev. R Palmer, in the Queensland Museum, Brisbane.

Only one fairly abundant infection has been seen, although blood films have been examined from several water-dragons from southern Queensland.

Morphology (Plate 2, Figs. 10–13)

Most of the parasites were elongate, very slender, curved forms, measuring 17–20 μ by 1·5 2 μ, enclosed in a delicate capsule. The nucleus was drawn out into a narrow band situated along one edge of the parasite for one-quarter to one-half of its length. The cytoplasm was free from granules (Fig. 12). Some extracellular but still encapsulated vermicules were seen, and one free form 22 by 2 μ (Fig. 13). A few unencapsulated trophozoites, about 12 by 3·5 μ,

with large nuclei and vacuolated cytoplasm, were present (Figs. 10 and 11). Normal cells measured 14–17 μ by 7·5–10 μ. Infected cells were slightly enlarged, measuring 16–19 μ by 9–11 μ; their nuclei were not distorted in any way, merely pushed to one side.

This is the only haemogregarine reported from agamid lizards in Australia. It seems to be distinct in the slenderness of its vermicules and the narrowness of their nuclei from those of other Australian lizards, and from three species which have been described from agamid lizards elsewhere, namely *Hg. thomsoni* Minchin, 1908, from *Agama tuberculata* from India, *Hg. agamae* Laveran & Pettit, 1909, from *Agama colonorum* from Senegal, and *Hg. percomsi* Shortt, 1922, from *Agama nupta* from Persia. It resembles *Hg. gracilis* Wenyon from a Sudanese skink.

I have pleasure in naming this species after the Rev. R. Palmer, who has provided me with many interesting blood films from native animals.

Family Scincidae

Haemogregarina tiliquae Johnston & Cleland

Haemogregarina tiliquae Johnston and Cleland, 1912, p. 484.

Host.—Tiliqua scincoides (Shaw).

Distribution.—Sydney.

Johnston and Cleland (1912) described this species from a blue-tongued lizard obtained near Sydney in October, 1910. The parasites were from 13–20·5 μ in length by 4 μ in the broadest part of the body. The cytoplasm stained a deep blue with Giemsa, and the nucleus varied in appearance in different specimens, sometimes being broad and reticular, sometimes granular and dispersed in one or more bands. The infected red cells were slightly distorted, their nuclei pushed to one side and elongated. In addition to these older stages, they observed numerous early forms which were more or less spherical, varying from 4–7 μ by 4·5 μ, and situated either in the lateral or polar positions. In an earlier stage, the bodies appeared as deep purple rings with clear centres.

Morphology (Plate 2, Figs. 14–17)

A moderately heavy infection was studied in films from a *T. scincoides* from Sydney (the type locality) sent to me by Mr. A. J. Bearup. The parasites agreed well with the original description. Two forms were present. The more abundant parasites were elongate, encapsulated, 14–16 μ by 3–4 μ, lying lateral to the host cell nucleus. A short narrow tail could be made out in some. The cytoplasm of the parasite stained blue in a Leishman-stained film and pinkish in a Giemsa-stained film. There was a medium-sized reticular nucleus, slightly posterior in position (Fig. 16). Two vermicules, 13–14 μ by 3 μ, with cytoplasm and nuclei like the intracellular forms, were also seen (Fig. 17).

The less abundant forms were short oval bodies, 8–11 μ by 4–6 μ, lying at one end of the red cell. A capsule was not detected around them. They stained heavily and some had a very large nucleus composed of thick strands of chromatin (Fig. 15). The cytoplasm was distinctly vacuolated. Some dark reddish rings with

clear centres were also seen in association with the small haemogregarines and appeared to be chromatin extruded from the parasite nucleus (Fig. 14).

Normal red cells measured 16–20 μ by 10–12 μ, infected cells 19–23 μ by 10–15 μ. The host cell nucleus was slightly elongated and flattened, and sometimes displaced laterally, but was not markedly distorted.

Haemogregarina hinuliae Johnston & Cleland

Haemogregarina (*Karyolysus*) *hinuliae* Johnston and Cleland, 1910, p. 684. Type slide from *Sphenomorphus quoyii* from Milson I., in the Australian Museum, Sydney.

Host.—*Sphenomorphus quoyii* (Duméril & Bibron), formerly known as *Hinulia quoyii*, hence the name of the parasite.

Distribution.—Milson I., Hawkesbury R., N.S.W.; Innisfail, N. Qld.

Johnston and Cleland described this species from a rather scanty infection in a skink shot at Milson I. in March, 1909. The parasites were approximately 15–18 μ long by 5–6·5 μ broad. A tail was seen in one parasite. The nucleus was a rather small rounded structure situated near the posterior end. A closely investing capsule was present. One organism was seen which was partly extruded from the red cell; it was longer than the others, being 25 μ long by 5·5 μ wide at the anterior end and 3·6 μ at the posterior end just behind the nucleus. The infected cells were greatly enlarged along their longitudinal diameter. The haemogregarines were always orientated longitudinally in the host cell, and the nucleus of the latter was displaced to one pole, or extruded altogether.

Morphology (Plate 4, Figs. 1–3)

A moderate infection was found in 1 out of 2 *S. quoyii* trapped on the bank of the Johnstone R. at Innisfail. The parasites were very uniform in size and shape. The intracellular forms were encapsulated, measuring 17–19 μ by 5–6 μ in the doubled-up condition. When measured along the mid-line, the total length varied from 23 to 27 μ, and the maximum breadth was 5 μ at the anterior end. The cytoplasm of the anterior part of the body was finely granular, except at the tip, which was clear. The nucleus was small, composed of a number of coarse chromatin granules, and situated just anterior to the bend. Every parasite seen was lying lengthwise in the host cell, occupying nearly all the available space. What remained of the red cell cytoplasm stained normally, but the nucleus was very dark, elongate, or flattened, sometimes forming a cap over the end of the parasite (Figs. 1 and 2). One large free vemicule was found, measuring 28 μ by 6 μ in maximum breadth (Fig. 3). Normal red cells measured 14–17 μ by 7–8·5 μ; infected cells 21–24 μ by 7·5–9 μ.

Haemogregarina taeniolati, sp. nov.

Host.—*Sphenomorphus taeniolatus* (Shaw).

Distribution.—Sydney to Broken Bay, N.S.W.

Type.—Slide from *S. taeniolatus* from North Sydney, in the Queensland Museum, Brisbane.

Johnston and Cleland (1912) reported finding haemogregarines in films from *S. taeniolatus* sent to them by Dr. T. L. Bancroft, from Eidsvold. They did not describe the parasites, which may have been the species described here.

Morphology (Plate 4, Figs. 4, 4*a*, 5)

Parasites were found in 3 out of 30 *S. taeniolatus* from various localities between Sydney and Broken Bay. They were abundant in two lizards, and rather scanty in the third. The encapsulated vermicules were of medium size, 12–15 μ by 2·5–3·5 μ, situated in the long axis of the host cell, and usually displacing the nucleus laterally (Fig. 4). The capsule was thin, and sometimes difficult to distinguish. The cytoplasm stained pale blue, and sometimes contained reddish granules. The nucleus was small and circumscribed, or formed a loose reticular structure, sometimes dispersed as 2 or 3 bands across the body.

Free vermicules were readily found in lung and liver smears from one heavily infected lizard. They were elongate bodies, 19–23 μ long by 3 μ in breadth at the broader end, and tapering to about 2 μ at the narrower end. The cytoplasm stained a pinkish blue, and the granular nucleus was rounded or elongate. Numerous reddish granules were present on each side of the nucleus in some vermicules, appearing like particles of chromatin extruded into the cytoplasm. Some vermicules showed a differentiation of the cytoplasm into a pinkish cap at the anterior end and a short clear area at the tip of the tail (Fig. 4*a*).

Encapsulated vermicules and scanty trophozoites were present in the third lizard. The trophozoites measured 12–13 μ by 3–4 μ, with blue vacuolated cytoplasm and a medium-sized, granular or reticular, centrally placed nucleus (Fig. 5).

Only slight enlargement of the infected red cells occurred, normal cells measuring 13–16·5 μ by 7–9 μ, and infected cells 15–19 μ by 7–12 μ. The host cell nuclei were somewhat flattened and displaced, but were not otherwise distorted. The displacement of the nucleus was usually lateral, and terminal displacement was rare (Fig. 4), the infected cell then appearing like those harbouring *Hg. hinuliae*.

This species is distinguished from *Hg. hinuliae* by its smaller size, and in causing less distortion of the host cell. The hosts have different habits and habitats.

HAEMOGREGARINA CUNNINGHAMI, sp. nov.

Hosts.—*Egernia cunninghami* (Gray), *E. striolata* (Peters).

Distribution.—Sydney, Roslyn, N.S.W.; Inglewood, Eidsvold, Qld.

Type.—Slide from *E. cunninghami* from Sydney, in the Queensland Museum, Brisbane.

Morphology (Plate 4, Figs. 6–9)

These parasites were present in 3 out of 9 *E. cunninghami* from Sydney, in a slide made by the Rev. R. Palmer at Roslyn, and in both of two from Inglewood. Most of the parasites were elongate, well-stained, encapsulated vermicules lying lateral to the host nucleus. They measured 13–15 μ by 3–3·5 μ, possessed a

moderate-sized reticular nucleus situated near the posterior end, and blue cytoplasm, which sometimes contained a few fine granules (Fig. 8). Two free vermicules were found, 23 μ and 26 μ long by 4 μ at the anterior end, and tapering gradually to about 2 μ near the tip of the tail. Their cytoplasm contained numerous fine, pink granules (Fig. 9). Some unencapsulated trophozoites were present, measuring 13–16 μ by 3–5 μ, with a bulky reticular nucleus situated in the middle of the body, and a slightly vacuolated cytoplasm (Figs. 6 and 7). Slight enlargement of the red cells occurred. Normal cells of the lizard from which the type slide was obtained measured 15–19 μ by 8–9·5 μ; infected cells 17–21 μ by 9–9·5 μ; their nuclei were displaced laterally, but not distorted.

The parasites seen in *E. cunninghami* from Inglewood and in *E. striolata* from Eidsvold are tentatively placed here, but the infections were too scanty for useful study, and practically all the haemogregarines seen were trophozoites (Figs. 10 and 11).

Haemogregarina egerniae, sp. nov.

Hosts.—Egernia cunninghami (Gray), *E. striolata* (Peters).

Distribution.—Sydney; Eidsvold, Qld.

Type.—Slide from *E. cunninghami* from Sydney, in the Queensland Museum, Brisbane.

Morphology (Plate 4, Figs. 12 and 13)

Infections were found in 7 out of 9 *E. cunninghami* taken near Sydney, and in 1 out of 7 *E. striolata* from Eidsvold.

Most of the parasites were unstained, appearing as short, stout, pale objects, 8–10 μ by 3·5–5 μ, lying at one end of the red cell (Fig. 13). The actual shape of the parasite could not be made out owing to the thickness of the capsule, but it is assumed that they were vermicules. Some parasites, however, were unencapsulated and well stained, 5–8 μ by 2–3 μ, with relatively large, band-like, reticular nuclei and slightly vacuolated cytoplasm (Fig. 12). In one fairly abundant infection in an *E. cunninghami*, only about 4% of the parasites were well stained, but in the infection in *E. striolata* the majority were well stained, and only an occasional unstained form was found. No free vermicules were found. Normal cells measured 15–20 μ by 7·5–11 μ, the average of 15 measured being 18 μ by 9 μ; cells infected with unstained parasites measured 17·5–21 μ by 8–10 μ, the average of 15 being 19 μ by 9 μ. Their nuclei were not altered in appearance or position.

Haemogregarina obscura, sp. nov.

Host.—Egernia cunninghami (Gray).

Distribution.—Sydney.

Type.—Slide from *E. cunninghami* from Sydney, in the Queensland Museum, Brisbane.

Morphology (Plate 4, Figs. 14 and 15)

A very scanty infection was present in 1 out of 9 *E. cunninghami* taken near Sydney. The parasites appeared as oval unstained areas, 13–14 μ by 4–5 μ, closely applied to the nucleus of the red cell; their shape was obscured by the thick capsule. No asexual forms were found but the infection was too scanty for this negative finding to be significant. The infected cells were considerably enlarged, measuring about 24–25 μ by 11–13 μ. Their cytoplasm stained abnormally pink, and their nuclei were swollen, spongy-looking, and often fragmented (Fig. 15). This species may be close to *Hg. johnstoni*, a karyolytic species found in *Varanus* spp.

The differential features of the species from skinks are summarized in the following key:

KEY TO SPECIES OF HAEMOGREGARINES IN AUSTRALIAN SCINCIDAE

(Based on the appearance of the intracellular vermicule)

1. Causing marked enlargment of red cell ..... *obscura*
   Not causing marked enlargement 2
2. Short, broad, unstained; polar in host cell ..... *egerniae*
   Medium or long, well stained 3
3. Very long, broad, displacing the nucleus of the red cell terminally ..... *hinuliae*
   Usually displacing nucleus laterally 4
4. Long, fairly broad, with posteriorly placed nucleus ..... *cunninghami*
   Of medium length and breadth, with nearly central nucleus 5
5. With moderately large reticular nucleus ..... *tiliquae*
   With small granular nucleus ..... *taeniolati*

Family Varanidae

HAEMOGREGARINA VARANICOLA Johnston & Cleland

Haemogregarina (*Karyolysus*) *varanicola* Johnston and Cleland, 1910, p. 683. Type slide from *Varanus varius varius* from Bingara, N.S.W., in the Australian Museum, Sydney.

Hosts.—*Varanus varius varius* (Shaw), *V. varius belli* (Duméril & Bibron), *V. tristis orientalis* Fry, *V. gouldii* (Gray).

Distribution.—Victoria; Bingara, Myall Lakes, N.S.W.; West Burleigh, Inglewood, Mt. Nebo, Fraser I., Eidsvold, Townsville, Innisfail, Qld.

Johnston and Cleland described this species from a scanty infection in blood films from a goanna taken by Mr. A. R. McCulloch near Bingara in December 1909. The authors stated that they were relatively large, about 13·5 μ by 4 μ. The nucleus was either a broad band or a rounded structure lying towards one end. In forms in which the capsule was clearly seen, the end of the parasite was tucked under to form a tail. These measured 16·7–18 μ along the mid-line. No distortion of the host cell was noted, except slight enlargement and displacement of the nucleus to one side.

What was probably the same parasite was recorded by Gilruth (1910) in 1 of 2 *V. v. varius* taken in the northern part of Victoria. Gilruth described and

figured it, but refrained from giving it a specific name, because the literature dealing with the species of haemogregarines already named from *Varanus* spp. in other parts of the world was not available to him.

Breinl (1913) recorded the presence of *Hg. varanicola* in 3 out of 5 *V. v. varius* in Townsville. He found free forms, some with a dividing nucleus, in smears from the lung of a heavily infected animal. Some parasites showed a second, small, chromatic mass near the anterior end. Breinl dissected ticks, *Aponomma trimaculatum*, taken from an infected lizard, and found numerous haemogregarines in the gut contents. He considered that these ticks probably acted as intermediate hosts, although no developmental stages were seen.

Morphology (Plate 5, Figs. 1–3)

Infections have been seen in varanids from various localities in Queensland and New South Wales. Usually a mixed infection was found, which made it difficult to associate the trophozoites and vermicules correctly. One *V. t. orientalis* from Innisfail had an abundant infection of intracellular vermicules, which agreed closely with the parasites found in the type slide (Fig. 1). They were remarkably uniform in size and appearance, measuring 14–15 μ by 2–3 μ, with a fairly large dense nucleus, usually composed of coarse granules, and situated posteriorly. The cytoplasm stained pale blue and was usually free from granules (Fig. 2). Free vermicules were found in moderate numbers and they were also very uniform in appearance, measuring 21–23 μ by 2 3 μ (average of 9 was 22 μ by 2·5 μ), with a fairly dense oblong nucleus and pale blue cytoplasm. Occasionally, a few pink granules were present but in many the cytoplasm appeared quite homogeneous (Fig. 3). No trophozoites were found in these films. Normal cells measured 14-17 μ by 7–9 μ; infected cells 15·5–19 μ by 8–9·5 μ; their nuclei were sometimes slightly flattened and were usually displaced laterally.

HAEMOGREGARINA GOULDII Johnston & Cleland

Haemogregarina (*Karyolysus*) *gouldii* Johnston and Cleland, 1912, p. 488. Type slide from *Varanus gouldii* from Eidsvold, in the Australian Museum, Sydney.

Host.—*Varanus gouldii* (Gray).

Distribution.—Eidsvold, Qld.

Johnston and Cleland described this species from films from two specimens of *V. gouldii* taken by Dr. T. L. Bancroft in October 1910 and March 1911.

Morphology (Plate 5, Figs. 4 and 5)

The authors stated that most of the haemogregarines were rather thin elongate bodies about 20 μ by 2·5 μ, investing the nucleus of the red cell very closely. The parasite nucleus was dense and deeply staining and numerous chromatinic granules were dispersed throughout the cytoplasm. The parasites had a detrimental effect on the nucleus of the host cell, causing it to become considerably elongated. Other kinds of haemogregarines were also present.

This species was not seen in any of the 45 varanids examined, and the illustrations (Figs. 4 and 5) are taken from the type slide, from which the

following additional information was obtained. The encapsulated vermicules measured 18–21 μ along the mid-line by 2–2·5 μ. The nucleus was narrow, dense, sometimes granular, and the cytoplasmic granules varied from fine to coarse dots or short rods. Normal cells measured 13·5–15·5 μ by 7–8·5 μ and their nuclei 3–5 μ by 2–3 μ; infected cells measured 18–21 μ by 8–9 μ and their nuclei 9–11 μ by 1·5–2·5 μ.

Haemogregarina johnstoni, sp. nov.

Hosts.—*Varanus varius varius* (Shaw), *V. gouldii* (Gray).

Distribution.—Mt. Nebo, Inglewood, Fraser I., Eidsvold, Qld.

Type.—Slide from *V. v. varius* from Mt. Nebo, in the Queensland Museum, Brisbane.

This species is present in low density in the type slide of *Hg. gouldii*, and was figured by Johnston and Cleland (1912, plate XVI, fig. 6) as an extreme example of red cell distortion by that species. It has been seen in pure infections, or mixed with *Hg. varanicola*, in 14 out of 45 varanids. It is named in honour of Professor T. H. Johnston, to whose efforts much of our knowledge of the parasites of native animals is due.

Morphology (Plate 5, Figs. 14–16)

Trophozoites were well-stained, oval, unencapsulated bodies, 10–12 μ by 3–6·5 μ, with medium-sized, granular or reticular, centrally placed nucleus, and vacuolated cytoplasm (Figs. 14 and 15). Encapsulated vermicules measured 12–15 μ by 3·5–5 μ, with granular nuclei which sometimes stained very faintly (Fig. 16). Both forms lay near the centre of the red cell, closely pressed against its nucleus, which was swollen, elongated, and stained abnormally, appearing much redder than normal. The red cell cytoplasm was thin, pale, and fragile. Normal red cells measured 14·5–18·5 μ by 8–10 μ and their nuclei 4–6 μ by 3–4 μ; cells containing vermicules measured 21–29 μ by 11–17 μ and their nuclei 9–14 μ by 3–4 μ.

Associated Exoerythrocytic Stages (Plate 5, Figs. 17 and 18)

Scanty X and Y schizonts were found in the liver and X schizonts in the heart of one *V. v. varius*. Y schizonts were found in the liver of another. They were not seen in sections of lung, lymph gland, or intestine. The smallest X schizonts were ovoid bodies about 10 μ in diameter and about 15–20 μ long; they were studded with large dark nuclei. Larger, nearly mature X schizonts measured about 30 μ in diameter and contained numerous nuclei. It appeared that at least 50 micromerozoites were formed (Fig. 17). An X schizont in a heart section measured 25 by 20 μ, with numerous nuclei, but was not yet divided into merozoites. The Y schizonts appeared as ovoid bodies, about 20 by 15 μ by 15 μ thick, containing 1–5 macromerozoites, each with a small dense nucleus (Fig. 18). Unfortunately, both these lizards had a mixed infection with *Hg. varanicola* and *Hg. johnstoni*, so that it is impossible to say to which species the schizonts belong.

Haemogregarina breinli, sp. nov.

Hosts.—*Varanus varius varius* (Shaw), *V. tristis orientalis* Fry, *V. gouldii* (Gray).

Distribution.—Eidsvold, Innisfail, Qld.

Type.—Slide from *V. t. orientalis* from Innisfail, in the Queensland Museum, Brisbane.

A moderately abundant infection was found in slides made by Dr. J. L. Harrison and Miss M. L. Emanuel, of the Institute's Field Station, Innisfail, from a live lizard at Innisfail. It is named in honour of Dr. A. Breinl, who made some of the earliest observations on the parasites of native animals.

Morphology (Plate 5, Figs. 6, 7, ?8 and 9)

Encapsulated vermicules measured 14–16 μ by 1·5–2·5 μ, and had moderately large fairly dense nuclei situated near the mid-point or slightly posterior to it. The cytoplasm stained pink or pinkish blue with Giemsa's stain and sometimes contained fine red granules (Fig. 6). Free vermicules measured 14·5–18 μ by 1·5–2·5 μ, with nucleus and cytoplasm like those of the intracellular forms (Fig. 7). The average of 15 measured was 16 by 2 μ. Normal cells measured 14–17 μ by 9–11·5 μ; infected cells 16–19 μ by 8·5–10 μ; their nuclei were sometimes slightly flattened and displaced laterally, but were sometimes unaltered. Some small trophozoites, measuring 8–10·5 μ by 2–3 μ, with large reticular nuclei and vacuolated cytoplasm, were also present (Figs. 8, 9); but, as there was another species in the slides, they cannot be identified with certainty.

Similar parasites were seen in small numbers in slides from a *V. v. varius* and a *V. gouldii* from Eidsvold.

Haemogregarina gilruthi, sp. nov.

Host.—*Varanus tristis orientalis* Fry.

Distribution.—Innisfail, N. Qld.

Type.—Slide from *V. t. orientalis* from Innisfail, in the Queensland Museum, Brisbane.

A fairly heavy infection was found in slides made from a live lizard at Innisfail. It is named in honour of Dr. J. A. Gilruth, who was one of the first to study the parasites of native animals.

Morphology (Plate 5, Figs. 10–12, ?13)

Encapsulated vermicules were plump bodies with rounded ends, measuring 13–15 μ by 3–4·5 μ, with a fairly large nucleus placed near the centre or slightly posterior to it, and composed of coarse strands of chromatin. The cytoplasm stained pinkish and frequently contained two pink bodies, about 1 μ in diameter, lying one on each side of the nucleus but quite separate from it (Fig. 10). In some parasites, only one pink body was seen, and occasionally one would be replaced by a group of fine granules. Free vermicules measured 19–23 μ by 3–4·5 μ, the average of 15 being 21 μ by 3·5 μ. Some were fairly uniform in diameter (Fig. 12) but usually one end was distinctly broader and longer than the other, which

sometimes tapered away rather suddenly (Fig. 11). The pink paranuclear bodies were clearly seen in nearly every vermicule examined. Trophozoites (Fig. 13) were present in small numbers and those which were thought to belong to this species measured 12–15 μ by 3–4·5 μ, but, as *Hg. breinli* was also present, it was not possible to decide definitely which trophozoites should be associated with the large vermicules. Normal red cells measured 14–17 μ by 9–11·5 μ; cells infected with vermicules measured 15·5–20 μ by 8·5–11·5 μ. It was noted that the trophozoites caused more change in the shape of the red cell than the vermicules.

Relationships

Haemogregarines have been described from *Varanus* spp. in Africa. The oldest name is *Hg. varani* Laveran, 1905, from *V. niloticus*, from Pretoria. These haemogregarines varied from small rounded or oval bodies 3 4 μ in length to elongate vermicular forms 14 by 3 μ. The total length of the vermicules was about 28 μ. They did not distort the host cell, except to displace the nucleus in some cases (Laveran 1905). This account was enlarged by Laveran and Pettit (1909*b*), who reported finding cysts with macromerozoites and others with micromerozoites in the liver. The non-karyolytic forms which have been described as *Hg. varanicola* resemble *Hg. varani*, but their free vermicules are considerably smaller.

Nicolle and Comte (1906) described *Hg. borreli* from *V. griseus*, from south Tunisia. The authors stated that it was morphologically very different from *Hg. varani*, without specifying any particular difference. The majority of the parasites described as *Hg. borreli* were small or medium-sized, usually lying lateral to the nucleus, and not distorting the host cell. However, some parasitized cells were greatly distorted, with pale cytoplasm and more or less elongated or fragmented nuclei. The authors regarded these as containing degenerating parasites, but it seems possible that they were dealing with a mixed infection of *Hg. varani* and a species causing karyolysis, for which the name *Hg. borreli* is valid. *Hg. johnstoni* is the only Australian species resembling *Hg. borreli*, but it differs from it in being smaller, and in causing more swelling of the red cell.

Wolbach (1914) described a third species, *Hg. toddii*, from *V. niloticus* from Gambia. He found micro- and macrogametocytes and vermicular forms in the peripheral blood, and two kinds of schizonts in endothelial cells in organs, particularly in the lungs and liver. One kind produced eight merozoites and the other 64 much smaller merozoites.

Key to the Species of Haemogregarines in Australian Varanidae

(Based on the appearance of the intracellular vermicule)

1. Causing marked enlargement of the red cell ..... *johnstoni*
   Not causing marked enlargement 2
2. Very long, slender forms causing marked elongation of the host cell nucleus .. *gouldii*
   Forms of medium length, not causing as much alteration of the host cell nucleus 3
3. Slender forms ..... *breinli*
   Fairly broad forms 4
4. With posteriorly placed nucleus and relatively clear cytoplasm ..... *varanicola*
   With nearly centrally placed nucleus and well-defined cytoplasmic inclusions .... *gilruthi*

Haemogregarines of Snakes

Family Boidae

Several species have been described from pythons from Africa, Asia, and Australia. These include the following:

Haemogregarina pythonis (Billet, 1895) in *Python reticulatus* from Indo-China.
Hg. pococki Sambon & Seligmann, 1907, in *P. molurus* from India.
Hg. shattocki Sambon & Seligmann, 1907, in *Morelia spilotes spilotes* from Australia.
Hg. robertsonae Sambon, 1909, in a python from Gambia.
Hg. sebai Laveran and Pettit, 1909, in *P. sebai* from Senegal.
Hg. amethystina Johnston, 1909, in *Liasis amethystinus kinghorni* from Port Curtis, Qld.
Hg. moreliae Johnston, 1909, in *M. s. variegata* from Abrolhos Is., W. Aust.
Hg. megalocystis Gilruth, Sweet, & Dodd, 1910, in *M. s. variegata* from Australia.
Hg. fuscus Lewis, 1913, in *L. fuscus* from the Northern Territory.
Hg. franchinii Yakimoff & Rastegaeva, 1930, in *P. molurus* from India.
Hg. phisalix Yakimoff & Rastegaeva, 1930, in *P. molurus* from India.

Of those recorded from Australia, *Hg. pythonis*, as redescribed by Prowazek (1912) from the type host, and *Hg. megalocystis* are well-defined species. The status of the others is somewhat confused. *Hg. pococki*, *Hg. shattocki*, *Hg. amethystina*, and *Hg. moreliae* were placed as synonyms of *Hg. pythonis* by Johnston (1912). However, it seems probable that several species occur in pythons. Four distinctive types of infection have been seen in Queensland. They will be described below, and an attempt made to allot them to the specific names already published.

Hg. sebai was described as a rather slender vermicule with slightly curved ends. Multiplication cysts containing 2–8 merozoites were found in the liver. This species may be close to *pythonis*, but may be distinct. Little is known about *Hg. robertsonae*, and the descriptions of *Hg. franchinii* and *Hg. phisalix* given by Yakimoff and Rastegaeva (1930) are not available here.

Haemogregarina pythonis (Billet)

Danilewskya pythonis Billet, 1895, p. 30.
Haemogregarina pythonis (Billet, 1895), Prowazek, 1912, p. 259.
Haemogregarina shattocki Sambon and Seligmann, 1907, p. 284, in part.
Haemogregarina amethystina Johnston, 1909*a*, p. 258, in part.

Hosts.—*Python reticulatus* L. (type host), *Morelia spilotes variegata* Gray, *M. spilotes spilotes* (Lacépède), *Liasis amethystinus kinghorni* Stull, *Chondropython viridis* (Schlegel).

Distribution.—Indo-China (type locality); Sumatra; Queensland; New South Wales.

In the original description, Billet stated that the parasites were gregarine-like and caused enlargement of the red cell. However, in the figure of an elongate

doubled-up parasite the red cell is not markedly enlarged and the nucleus is not distorted. Billet also figured a large, more or less oval body, with granular contents, which was considered to be "the cytocyst of the schizogonic stage".

Prowazek (1912) figured elongate, slender, encapsulated parasites in the type host from Sumatra. Sometimes the host cell was enlarged and its nucleus displaced, but sometimes there was no marked alteration. Two much shorter sausage-shaped parasites, with small nuclei, were also illustrated. These were considered to be female gametocytes. Free vermicules were figured. They were elongate, with relatively small central nuclei and with one end blunt and the other pointed. Schizonts were found in the lung. It is not certain that the species described by Billet was the same as that studied by Prowazek, but the latter's definition of *Hg. pythonis* has been accepted here.

Laveran (1908) studied the infections present in three *M. s. spilotes* from Australia. He found relatively slender elongate forms, sometimes club-shaped, encapsulated, with small round nuclei, causing slight enlargement of the red cells and displacement, but not distortion, of their nuclei. Free vermicules were found, measuring 11 - 20 μ, with elongate cylindrical nuclei and clear cytoplasm. Schizonts were found in the capillaries of the lung. He identified the parasites as *Hg. shattocki,* but his figures are very similar to those of *Hg. pythonis* as redescribed by Prowazek (1912).

Blood films of 19 *M. s. variegata* have been examined from various localities in south Queensland and New South Wales, haemogregarines being found in nine. There were considerable differences between the parasites present in different snakes, but in four the haemogregarines conformed fairly well to *Hg. pythonis*. Free vermicules were present in moderate numbers in one of them.

Morphology (Plate 6, Figs. 1–4)

Intracellular vermicules were slender, 12–15 μ by 2–3·5 μ, and usually slightly curved at each end within the capsule, which was clearly visible. The nucleus was small, oblong, dense in some, granular in others, and usually occupying the full width of the parasite, but not bulging outward. The cytoplasm was clear and faintly stained. Free vermicules were slender bodies with pointed ends. They measured 16–19 μ by 1·5–2 μ, with similar nuclei and cytoplasm to the intracorpusclar forms in the same film (Fig. 3). Normal red cells measured 13–19 μ by 8–10 μ; infected cells 17–20 μ by 8·5–10 μ; their nuclei were sometimes displaced but not distorted. Sections of liver and lung were obtained from one of these snakes, but no schizonts were found. Parasites seen in a film from a *C. viridis*, collected on Cape York Peninsula by Mr. J. L. Wassell, are tentatively placed here, although they are larger than the typical forms (Fig. 4).

HAEMOGREGARINA POCOCKI Sambon & Seligmann

Haemogregarina pococki Sambon and Seligmann, 1907, p. 283.

Hosts.—Python molurus (type host), *Morelia spilotes variegata* Gray.

Distribution.—India (type locality); Mt. Nebo, Qld.

Sambon and Seligmann described the parasites as club-shaped, 14–16 μ long, with large, oval, coarsely granular nuclei. They did not cause much alteration of the host cell, except displacement of the nucleus.

Morphology (Plate 6, Figs. 5 and 6)

Parasites in one *M. s. variegata* resembled the figures of Sambon and Seligmann fairly closely (Fig. 5). The encapsulated vermicules measured 13–15 μ by 3–4 μ. One end was slightly recurved and the other blunt. The nucleus was 3–5 μ long, composed of thick strands of chromatin filling the whole width of the parasite. A few trophozoites were seen, with large open nuclei and vacuolated cytoplasm (Fig. 6). Normal cells measured 15·5–18 μ by 8–11 μ; infected cells 18–23 μ by 8 5–9·5 μ; the nucleus was usually displaced but was not distorted.

HAEMOGREGARINA SHATTOCKI Sambon & Seligmann

Haemogregarina shattocki Sambon and Seligmann, 1907, p. 284.
Haemogregarina amethystina Johnston, 1909*a*, p. 258, in part.

Hosts.—Morelia spilotes spilotes (Lacépède) (type host), *M. spilotes variegata* Gray, *Liasis amethystinus kinghorni* Stull, *?Pseudechis guttatus* de Vis, *?Notechis scutatus* (Peters).

Distribution. New South Wales (type locality); Mt. Nebo, Brisbane, Port Curtis, Qld.

The authors described the species from what was evidently a mixed infection in a diamond python. Some of their figures were very like *Hg. pythonis*, and their descriptions included slender forms falling in the range of measurements of that species. The larger parasites, to which the name should now be restricted, were larger than *Hg. pococki*, and had remarkably large nuclei, measuring as much as 9 by 4 μ.

Morphology (Plate 6, Figs. 7–11, 14, 15)

Parasites were found in a carpet snake which was kept in captivity for 20 months. Films made on three occasions consistently showed the same sorts of haemogregarines. At the first examination, parasites were numerous, mostly oval or sausage-shaped, 8–14 μ by 3–4 μ, with a particularly voluminous nucleus. The chromatin often appeared to be arranged in thick strands wound round the body. A capsule was not detected (Fig. 9). Six months later fewer parasites were seen. They were similar to those described but also included stout encapsulated forms, 13·5–16·5 μ by 3·5 μ, with large wedge-shaped nuclei, 7–11 μ long, extending across the whole width of the parasite, similar to that shown in Figure 8. Ten months later parasites were still present in about the same density as at the previous examination, both trophozoites (Fig. 7) and vermicules being present. Normal cells measured 13–18 μ by 7–11 μ; infected cells 16–19 μ by 7·5–11 μ; the nuclei were usually compressed and displaced laterally. Very similar parasites were present in a single film given to me by Professor J. F. A. Sprent, Veterinary School, University of Queensland.

After the captive snake died, sections were made of several organs, and schizonts of two kinds were found quite readily in liver, lymph gland, and spleen, being most numerous in the liver. Unfortunately the lung was not preserved. The X schizonts were ovoid structures, with rather foamy cytoplasm, containing from one to numerous large darkly stained nuclei (Fig. 10). The fully developed schizonts were about 30 by 15 μ in section, and 15–20 μ in thickness. It was difficult to count the merozoites, but certainly more than 20 were formed (Fig. 11). These were slender elongate bodies, with very dense rather oblong nuclei.

The Y schizonts were ovoid bodies about 20 by 15 μ (Fig. 15), containing a few large merozoites. Usually only 2 or 3 merozoites were visible in a longitudinal section, but the maximum number noted in a cross section was 5, and it seems likely that 6 or 8 may be formed. The merozoites were about 15 by 3–4 μ, with round dense nuclei. These schizonts were found most frequently in association with large accumulations of pigment. Sometimes three or four schizonts appeared to be contained in a mass of macrophages loaded with pigment (Fig. 14). It is interesting to note that Laveran and Pettit (1909*a*) found *Hg. sebai* in large pigmented cells from the lung of *Python sebai*.

Haemogregarina amethystina Johnston

Haemogregarina amethystina Johnston, 1909*a*, p. 258, in part. Type slide from *Liasis amethystinus kinghorni* from Port Curtis, in the Australian Museum, Sydney.

Hosts.—*Liasis amethystinus kinghorni* Stull, *Morelia spilotes variegata* Gray.

Distribution.—Port Curtis, Mt. Mitchell, Qld.

Examination of the type slide, which is in good condition, suggests that a mixed infection is present. Elongate slender parasites, which are easily found and which were figured by Johnston, are very similar to *Hg. pythonis*. Broader forms with large nuclei are similar to *Hg. shattocki*. Some short broad forms with small nuclei were thought by Johnston to be sporonts in which the tail was not visible. I would restrict the name *amethystina* to these forms (Figs. 19 and 20 of Johnston), which have been seen in an apparently pure infection in a python collected by Dr. Dorothea F. Sandars, of this Institute, at Mt. Mitchell in southern Queensland.

Morphology (Plate 6, Figs. 12 and 13)

The parasites were numerous in the snake from Mt. Mitchell, and very uniform in conformation, appearing as sausage-shaped encapsulated forms measuring 13–14 μ by 3–4 μ. The cytoplasm was clear blue and the nucleus relatively small, elongate, or wedge-shaped and sometimes granular. Occasionally it could be clearly seen that the actual parasite was much longer than its capsule and possessed a relatively long thick tail doubled-up beside it, making the nucleus appear to extend only half way across the body (Fig. 13). The total length was about 20 μ, by 3 μ wide at the broader end and 1 μ at the narrower end. A striking feature, under some conditions of staining, was the appearance of a bright red area at each end of the majority of parasites, suggesting a layer of eosinophilic substance between the parasite and its capsule (Fig. 12). This appearance was not

seen in the type slide, but some parasites had fine red stippling over them, not seen in *Hg. pythonis* or *Hg. shattocki* in the same slide. Normal cells measured 14–17·5 μ by 7–9 μ; infected cells 15·5–19 μ by 8–10 μ; the nucleus was usually slightly elongated or flattened and displaced laterally.

Haemogregarina moreliae Johnston

Haemogregarina moreliae Johnston, 1909*b*, p. 404. Type slide from *Morelia spilotes variegata* from Abrolhos Is., in the Australian Museum, Sydney.

Host.—*Morelia spilotes variegata* Gray.

Distribution.—Abrolhos Is., W. Aust.

Morphology

It was described from films made by Dr. J. B. Cleland. The parasites were large, occupying nearly all the available space in the red cell, but not distorting it, except by displacing the nucleus. Crescentic forms were uncommon, most being club-shaped encapsulated "sporonts", varying from 10–19 μ by 1·5–6 μ. The largest forms measured 20 by 4 μ and 17·5 by 6 μ. The type slide has faded but the parasites are larger than any seen in recent material. Johnston (1909*b*) thought that this species might be the same as *shattocki*, and, in 1912, he placed both as synonyms of *pythonis*. However, it seems best to reserve judgment on this point until fresh material is available from the type locality.

Haemogregarina megalocystis Gilruth, Sweet, & Dodd

Haemogregarina megalocystis Gilruth, Sweet, and Dodd, 1910, p. 234.

Host.—*Morelia spilotes variegata* Gray.

Distribution.—Australia, the exact locality being unknown.

This species was described from an abundant infection in a snake which died in the Melbourne Zoological Gardens. It has not been reported since.

Morphology

The authors described the parasites as lying in the centre of the red cell alongside its nucleus, and varying in size from 13 by 4 μ up to 18·6 by 5·6 μ. No schizonts were seen. The body was fairly homogeneous, somewhat granular, and stained blue, often faintly at one end. The nucleus was generally small, spherical, usually central, pinkish, with more deeply stained granules. The capsule was rarely seen, but parasite and host nucleus were often surrounded by a faint zone, probably of degenerated stroma. The infected host cells were always greatly enlarged, measuring about 35–55 μ by about 20 μ, and their cytoplasm was extremely tenuous and pale. The host cell nucleus was longer, usually wider, and stained more deeply than normal.

I have not seen this species. It is evidently like *Hg. johnstoni* (Plate 5, Figs. 14–16) and *Hg. bancrofti* (Plate 8, Figs. 5 and 6).

Haemogregarina fuscus Lewis

Haemogregarina fuscus Lewis, 1913, p. 137.

Host.—*Liasis fuscus* Peters.

Distribution.—Northern Territory.

Lewis described this species from two heavy infections found in snakes obtained in coastal country about 100 miles from Darwin. It does not appear to have been reported since.

Morphology

The author described forms which were slender, almost cylindrical, with clear cytoplasm and small nuclei often not occupying the full width of the parasite. These were regarded as young forms. More-developed haemogregarines were stouter and blunter and their nuclei larger and subterminal, generally reaching both borders, and disposed to assume mitotic figures. The cytoplasm was granular. Others, which were considered still more advanced, showed pinkish cytoplasm and a much larger nucleus, irregular in shape, and seldom mitotic. All the infected cells were enlarged, and their nuclei displaced. Neither cytoplasm nor nuclei stained normally. Sections of the liver showed scanty schizonts situated near the capsule.

I have seen a single parasite in a *M. s. variegata,* which might belong to this species. It also resembles *Hg. megalocystis,* but does not appear to adhere to the red cell nucleus in the characteristic way shown in Gilruth's figures. The schizonts figured by Lewis look like the X schizonts found in the liver of *M. s. variegata* infected with *Hg. shattocki.*

Key to Species of Haemogregarines in Australian Boidae

(Based on the appearance of the intracellular vermicule)

1. Causing marked enlargement of red cell 2
   Causing slight enlargement of red cell 3
2. Parasite adhering to red cell nucleus ..... *megalocystis*
   Not adhering to red cell nucleus ..... *fuscus*
3. Short to medium, slender, with nearly centrally placed nucleus ..... *pythonis*
   Not as above 4
4. Long, broad, filling red cell, with reticular nucleus ..... *moreliae*
   Of medium length 5
5. Tightly encapsulated forms, with nucleus not extending across whole apparent width, sometimes with eosinophilic caps ..... *amethystina*
   Nucleus extending across whole width 6
6. Broad, with very large nucleus ..... *shattocki*
   Fairly broad, with medium-sized nucleus ..... *pococki*

Family Colubridae

Haemogregarina dendrophidis Johnston & Cleland

Haemogregarina (*Karyolysus*) *dendrophidis* Johnston and Cleland, 1910, p. 680. Type slide from *Dendrophis punctulatus* from south Queensland, in the Australian Museum, Sydney.

Host.—Dendrophis punctulatus (Gray).

Distribution.—South Queensland.

This species was described from a film made by Dr. T. L. Bancroft in December, 1907. There were numerous parasites, which varied in size from delicate thin crescents with pointed ends to stout sausage-shaped forms which displaced the host cell nucleus. These larger forms were 18 μ in total length measured along the mid-line, were encapsulated, and possessed a distinct tail. The cytoplasm frequently showed large deep blue granules scattered about the posterior end and around the nucleus, but they were absent from the anterior end. The large nucleus was situated near the bend. One parasite was found in the act of leaving the red cell. It was an elongate slightly curved body, with ends tapering gradually and fairly evenly; its cytoplasm contained chromatinic granules.

Morphology (Plate 7, Figs. 1–6)

Two scanty infections in green tree-snakes taken in south Queensland have been studied. In one snake, all the forms mentioned by Johnston and Cleland were present. The slender crescents measured 10–12 μ by 2 μ, and were frequently polar in position. Their cytoplasm stained pale blue and sometimes contained vacuoles. The nucleus was fairly large, occupying more than one-third of the total length. A capsule was not detected. They were sometimes found in erythroblasts (Fig. 4). Broader forms, up to 17 by 5 μ, with similar cytoplasm had a rather small granular or reticular nucleus. A capsule was not detected but some seemed slightly bent, as if a very delicate capsule might have been present. These appeared to be different from the trophozoites seen in another snake, which all had large reticular nuclei. The definitely encapsulated forms had a short tail tucked up beside them. They measured 15–17 μ by 3–4 μ. The cytoplasm was pale and uniform in appearance. The nucleus was moderately large, open reticular or granular, sometimes wedge-shaped, and situated near the bend (Fig. 5). One free vermicule was seen. It measured 25 by 3·5 μ and was rather evenly tapered towards each end, as remarked by the original authors. No chromatinic granules were noted in its cytoplasm and its nucleus was elongate and reticular (Fig. 6). One encapsulated vermicule was enclosed in a lymphocyte.

In the other infected snake, parasites were even less numerous and only unencapsulated forms, 11–15 μ by 3·5–4 μ, with large reticular nuclei, were found (Figs. 1–3).

Normal red cells measured 14–18 μ by 9–12 μ; cells infected with vermicules from 18·5–25 μ by 10–13·5 μ. Their nuclei were displaced laterally and sometimes slightly flattened and indented. Cells infected with trophozoites were usually broader than normal.

HAEMOGREGARINA CALLIGASTER Lewis

*Haemogregarina calligaster** Lewis, 1913, p. 137.

*Host.—Dendrophis calligaster** (Günther).

Distribution.—Northern Territory.

* Misspelt *"calligasger"* in the original.

Lewis stated that nearly all the individuals of this species of snake which he had examined were infected with haemogregarines, sometimes very heavily. He noted that the parasite did not greatly distort the host cell. It measured about two-thirds the length of the red cell. The parasite nucleus was small and variously placed and its cytoplasm usually clear.

The figures show rather slender parasites of medium length lying lateral to the host nucleus, and evidently encapsulated vermicules. Johnston (1916) thought that *calligaster* was probably a synonym of *dendrophidis* but it appears to be more like *mirabilis*. It is a smaller parasite than *dendrophidis* and causes less alteration of the host cell.

Haemogregarina mirabilis Castellani & Willey

Haemogregarina mirabilis Castellani and Willey, 1904, p. 86.

Hosts.—*Natrix piscator* (type host), *N. mairii* (Gray), *?Boiga fusca* (Gray).

Distribution.—Colombo, Ceylon (type locality); Bombay, India; ?Townsville, Brisbane, Qld.

This species was described by Castellani and Willey as V-shaped, about 12 μ long, and staining a uniformly blue colour, with dense nucleus lying near the anterior pole. In fresh preparations active vermicules were seen; in stained films they appeared pale blue, with a dense red-blue nucleus. The figures show a slender, elongate, encapsulated parasite, lying lateral to the red cell nucleus, and causing slight enlargement of the cell but not distorting its nucleus.

Ball (1958) found 50% of *N. piscator* taken near Bombay to be infected with *Hg. mirabilis*. Schizogony occurred in the lung and developmental stages were found in leeches, *Hirudinaria* sp., which fed readily on the snakes. No stages were found in trombiculid mites from the infected snakes.

The haemogregarines found by Breinl (1913) in 1 out of 2 *B. fusca* (syn. *Dipsadomorphus fuscus*) at Townsville are tentatively placed here. The parasites measured on the average 17 by 5 μ and lay lateral to the host cell nucleus. Normal cells measured 15·5–17·5 μ by 10–14 μ; infected cells 17·5–20 μ by 13·4–17·05 μ. The nucleus of the red cell was only slightly, if at all, displaced by the parasite. The figures published by Breinl are more like *Hg. mirabilis* than any other haemogregarine seen in colubrid snakes in Australia.

Haemogregarines found in the blood of 1 out of 4 *N. mairii* taken near Brisbane, have been assigned to this species, because most of the parasites were very similar to those described by Castellani and Willey, except that they were usually longer; schizogony occurred in the lung as described by Ball, and also in the liver; and the genus *Natrix* has a continuous distribution from India to Australia.

Morphology (Plate 7, Figs. 7–12)

The commonest parasites seen were elongate encapsulated forms, lying lateral to the nucleus of the red cell, and measuring 14·5–16 μ by 2·5–3·5 μ. The cytoplasm was light blue in a film stained by Leishman's method, and the nucleus small, round, and dense or coarsely granular. The cytoplasm appeared free from granules

in this film but, in another made at the same time and stained by Giemsa's method, fairly fine reddish granules were present in practically unstained cytoplasm. Some extracellular, but still encapsulated, forms were seen (Fig. 10), and a few free vermicules. The latter measured 17–22 μ in length; one end was longer and broader, up to 4 μ in diameter, and the other was shorter, about 3 μ in diameter.

The smallest trophozoites were slender crescentic bodies, about 11 by 2 μ, with a large nucleus nearly central in position, and with vacuolated cytoplasm (Fig. 7). A few very broad forms, about 15 by 5 μ, with pale cytoplasm and a very open reticular nucleus, were also seen (Fig. 8). Neither of these appeared to be encapsulated. Normal cells measured 16–19 μ by 8·5–12 μ; cells containing vermicules 16–21 μ by 9–13 μ. The host cell nuclei were sometimes slightly flattened and displaced but were not otherwise affected.

Sections of liver, lung, kidney, intestine, and brain were examined and X and Y schizonts were found in the lung and liver, more readily in the former. One X schizont was found in the brain. It measured 30 by 15 μ in section and contained at least 25 merozoites, each about 6–7 μ long. The X schizonts in the lung were ovoid bodies, about 30–35 μ by 10–15 μ, studded with numerous large nuclei (Fig. 11). The Y schizonts were ovoid bodies, about 20–25 μ by 10–15 μ, containing 2–6 large merozoites (Fig. 12).

Other Haemogregarines from Related Snakes

Several other species of haemogregarines have been described from related snakes in various parts of the world. *Hg. viperina* Billet, from *Tropidonotus viperinus* from Algeria belongs to the group possessing karyolytic action (Billet 1904). The sausage-shaped parasite is closely applied to the red cell nucleus and even penetrates into it. The red cell is greatly enlarged and its cytoplasm filled with reddish granules. *Hg. lahillei,* which was described by Brumpt (1928) from *T. natrix* from Corsica, resembles *Hg. mirabilis* in a general way; whilst *Hg. bradfordi,* described by Sambon (1909) from *T. fasciatus* from North America, resembles *Hg. viperina* in causing enlargement of the host cell. Hoare (1918) described *Hg. tigrinae* from *N. tigrina* from Japan. It is an elongate species, which does not have a karyolytic action.

Haemogregarina stegonoti, sp. nov.

Host.—*Stegonotus plumbeus* (Macleay).

Distribution.—Innisfail, N. Qld.

Type.—Slide from *S. plumbeus* from Innisfail, in the Queensland Museum, Brisbane.

The description is based on a moderate infection seen in films from a snake taken by Dr. J. L. Harrison at Innisfail.

Morphology (Plate 7, Figs. 13–16)

Most of the parasites were elongate, club-shaped, 15–17 μ by 3·5–4·5 μ, with pale pink cytoplasm, and a moderate-sized, often wedge-shaped, granular nucleus

which was situated posteriorly. A distinct tail could be seen in many and a thin capsule was clearly seen (Fig. 15). Free vermicles measured 26–27·5 μ by 3·5 μ at the broader end and 2 μ at the narrower end. The cytoplasm was clear and the nucleus compact and granular (Fig. 16). Scanty trophozoites, about 16 by 3·5 μ, had blue vacuolated or homogeneous cytoplasm and rather large, open, granular nuclei (Figs. 13 and 14).

All these parasites caused considerable alteration of the host cells. Their nuclei were usually enlarged, spongy-looking, staining a decidedly paler tint than normal; and their cytoplasm was swollen and pale, and frequently showed separation into a fairly normal portion immediately surrounding the nucleus and parasite and a much paler portion lying peripherally (Fig. 15). Large clefts were seen in the cytoplasm of some cells. Although these large parasites had a definite karyolytic action, and usually lay with their concave side close to the red cell nucleus, there did not seem to be the intimate contact between parasite and nucleus which was noted in *Hg. johnstoni*. Normal cells measured 15–21 μ by 8–11 μ and their nuclei 5·5–7 μ by 3–3·5 μ; cells containing encapsulated vermicules 23–28 μ by 9–14 μ and their nuclei 7·5–11·5 μ by 3–4·5 μ.

Hg. stegonoti differs from other species found in colubrid snakes in causing considerable swelling of the red cell cytoplasm and nucleus.

Other Haemogregarines Present (Plate 7, Figs. 17 and 18)

A second species of haemogregarine was present in small numbers in the same films. The encapsulated vermicules were slender, 13·5–15 μ by 1·5–2 μ, with pale cytoplasm, and moderately large dense nuclei which were placed posteriorly. They caused elongation of the host cells, and displacement of their nuclei laterally or terminally (Fig. 18) but the cytoplasm and nucleus stained normally. A few small crescentic parasites, about 9 by 2·5 μ, with large granular nuclei, should probably be associated with the slender vermicules (Fig. 17). These forms are morphologically close to *Hg. aspidomorphi*, which is described below, and have been tentatively assigned to that species.

HAEMOGREGARINA BOIGAE, sp. nov.

Hosts.—*Boiga fusca* (Gray), *Dendrophis punctulatus* (Gray).

Distribution.—Brisbane, Innisfail, Qld.

Type.—Slide from *B. fusca* from Innisfail, in the Queensland Museum, Brisbane.

Morphology (Plate 8, Figs. 1 and 2)

Infections were studied in films made by Miss M. L. Emanuel from a *B. fusca* taken in a canefield near Innisfail, and in films from a *D. punctulatus* made by Dr. D. F. Sandars in Brisbane.

The parasites in *B. fusca* were encapsulated, rather short vermicules, 11–13 by 3 μ, with small or medium-sized, centrally placed, reticular nuclei, and uniform blue-staining cytoplasm. One end was often irregularly shaped, pointed in some, rounded in others. Polar caps staining bright red with Giemsa's stain were present

at one or both ends (Figs.1,*e*;2,*e*). These were not part of the organisms as they were sometimes separated quite clearly from it. A peculiar, greyish green rounded structure was associated with each parasite, one red-capped end of which usually protruded into it. It seemed to be continuous with the capsule and was thought to be the distended end of it. It has been called the grey globule. Parasitized cells were elongated as a rule but not much altered in width; their margins were poorly defined and cytoplasm paler than normal. The red cell nucleus was usually elongated, compressed, and displaced laterally; sometimes it was swollen and stained abnormally. Normal cells measured 17–22 μ by 8·5–11·5 μ; infected cells 20–24 μ by 8–11 μ. No free vermicules and no trophozoites were seen in the films from *B. fusca.*

A few much stouter parasites were also present in the type series. They measured 16–17 μ by 4–5 μ, the cytoplasm stained pinkish, and the nucleus was medium-sized, reticular. These appeared to have closely fitting capsules. The red cells containing these stout parasites were enlarged in both dimensions, and their nuclei were elongated and displaced laterally (Plate 8, Fig. 3). They evidently belong to a different species, possibly *Hg. stegonoti*, but were too scanty for useful study.

Numerous Y schizonts were found in sections of the liver, which was the only organ examined from this snake. They varied in section from 14 by 10 μ up to 23 by 18 μ, and contained 1–5 elongate palely stained merozoites, each about 12 μ long, with a small, round, densely stained nucleus (Plate 8, Fig. 4). As two species of haemogregarines were present in the blood, these schizonts cannot be allotted with certainty to *Hg. boigae.*

The same species was subsequently seen in films from *D. punctulatus*. The infection was heavy, but, owing to the fact that the snake had been kept frozen for several weeks, considerable changes had occurred in the blood and organs, and the peculiar appearances described here may be artifacts.

Very scanty trophozoites were present, measuring about 16 by 4–5 μ, with blue vacuolated cytoplasm and a broad, central, reticular nucleus. Encapsulated forms were abundant but no free vermicules were found, which was surprising, as almost all the red cells were laked, and the parasites appeared to be extracellular, though still partially surrounding the red cell nucleus. Many organisms were like those seen in *B. fusca*, with bluish cytoplasm, central nucleus, eosinophilic caps, and associated grey globule. Others, however, appeared to have split longitudinally, and two elongate cigar-shaped bodies were seen with their bases still attached to the grey globule. Sometimes they stained uniformly pink, but sometimes one or both contained a round red body, like a nucleus.

Fairly numerous immature X schizonts were present in the lung. They were relatively small ovoid bodies, the largest measuring 27 by 13 μ in longitudinal section. Typical Y schizonts were not found but the liver contained numerous macromerozoites, which appeared to be lying in the sinusoids, and were sometimes associated with clumps of pigment-laden macrophages.

KEY TO THE SPECIES OF HAEMOGREGARINES IN AUSTRALIAN COLUBRIDAE*

(Based on the appearance of the intracellular vermicule)

1. Long parasites causing considerable enlargement of the red cell ..... *stegonoti*
   Causing slight enlargement of the red cell 2
2. Short slender forms with eosinophilic caps and associated grey globule ..... *boigae*
   Not as above 3
3. Long, with moderately large posteriorly placed nucleus ..... *dendrophidis*
   Of medium length 4
4. With rather small nearly central nucleus ..... *mirabilis*
   With narrow dense nucleus in posterior half of body ..... *aspidomorphi*

* Insufficient data to separate *calligaster* from *mirabilis*.

Family Elapidae

HAEMOGREGARINA PSEUDECHIS Johnston

Haemogregarina pseudechis Johnston, 1909*b*, p. 406. Type slide from *Pseudechis porphyriacus* from Sydney, in the Australian Museum, Sydney.

Hosts.—*Pseudechis porphyriacus* (Shaw), *P. australis* (Gray).

Distribution.—Sydney; Eidsvold, Qld.

The parasites described by Johnston were encapsulated, slightly club-shaped, with a thicker anterior end, and tapering very gradually towards a blunt, slightly curved, posterior end. The nucleus was broad, band-like, and generally situated towards one or other end. The full-grown parasites measured 12–14 μ by 3–4 μ. The host cells were not distorted, but their nuclei were usually displaced laterally.

Morphology (Plate 8, Fig. 9)

The type slide has faded badly and parasites are difficult to find. However, enough were seen to establish their close similarity to some haemogregarines present in films from a *P. australis* from Eidsvold.

Encapsulated vermicules were present in moderate numbers. They measured 12·5–15 μ by 2·5–4 μ, and one end was longer than the other and ended in a recurved narrower portion. The cytoplasm was uniformly pale. The medium-sized nucleus formed a rather open, reticular, band-like structure which stained pale blue in these films (Fig. 9). Normal red cells measured 16·5–19 μ by 10–12·5 μ; infected cells 18–21 μ by 10–13 μ; their nuclei were displaced but not distorted.

HAEMOGREGARINA BANCROFTI Johnston & Cleland

Haemogregarina (*Karyolysus*) *bancrofti* Johnston and Cleland, 1912, p. 486.

Hosts.—*Pseudechis guttatus* de Vis, *P. australis* (Gray), *Notechis scutatus* (Peters).

Distribution.—Mt. Nebo, Eidsvold (type locality), Qld.

The authors described this species from films from *P. mortonensis* (a synonym of *P. guttatus*) and *P. australis* taken by Dr. T. L. Bancroft. The

haemogregarines measured 10–17 μ by 3·5 μ. The protoplasm of the parasite was very faintly stained, and at times large vacuoles were seen. The nucleus was sometimes near one end, sometimes central. It resembled *Hg. megalocystis* in its effect on the host cell, which was markedly enlarged, the cytoplasm being thin and dehaemoglobinized. The parasite lay about the middle of the red cell, its concavity partly surrounding the host cell nucleus, which did not seem to be detrimentally affected. The surrounding stroma, however, was markedly affected. A capsule was seen in one case. Normal cells measured 15–19 μ by 10–11 μ; some infected cells measured as much as 31·5 μ by 17·5 μ.

Morphology (Plate 8, Figs. 5 and 6)

A scanty infection was seen in a *P. guttatus*, a few parasites in a *P. australis*, both from the type locality, and a very scanty infection in an *N. scutatus* from Mt. Nebo. The vermicules appeared as unstained sausage-shaped bodies, measuring 11·5–15 μ by 4–5 μ, and usually lying close to the host cell nucleus. The internal structure could not be made out. The normal red cells measured 16–20 μ by 9–11 μ, and their nuclei 5–6·5 μ by 2·5–3·5 μ; infected cells 21–27 μ by 11–16 μ, with their more elongated nuclei 7–11 μ by 2·5–3 μ (Figs. 5 and 6).

HAEMOGREGARINA AUSTRALIS, sp. nov.

Host.—Pseudechis australis (Gray).

Distribution.—Eidsvold, Qld.

Type.—Slide from *P. australis* from Eidsvold, in the Queensland Museum, Brisbane.

Morphology (Plate 8, Figs. 7 and 8)

Infections were seen in two *P. australis* from Eidsvold, one of which was also infected with *Hg. pseudechis* and *Hg. bancrofti*.

Most of the parasites appeared as oval, unstained, encapsulated vermicules, 10–13 μ by 4–5 μ, frequently polar in position (Fig. 8). There were also very scanty, small, well-stained parasites, about 9 by 3·5 μ, with vacuolated cytoplasm and centrally placed, granular nuclei, which were thought to be trophozoites. Normal cells measured 14–18 μ by 9–11 μ; infected cells 17–19 μ by 9·5–12 μ. Their nuclei were not distorted but were sometimes slightly displaced.

This species differs from *Hg. pseudechis* and *Hg. eidsvoldensis* in being a little shorter and broader, frequently in the polar position, and in having forms which do not become stained. It resembles *Hg. egerniae* in this respect. It differs from *Hg. bancrofti* in lacking a karyolytic action.

HAEMOGREGARINA EIDSVOLDENSIS, sp. nov.

Host.—Pseudechis australis (Gray).

Distribution.—Eidsvold, Qld.

Type.—Slide from *P. australis* from Eidsvold, in the Queensland Museum, Brisbane.

Numerous haemogregarines were found in a film from a *P. australis* made by Dr. T. L. Bancroft at Eidsvold. Rather delicate slender forms, with unusually small nuclei, were fairly abundant. These seem to be distinct from other known species and are described here as new. Some unstained forms, about 12·5 by 4 μ, which occupied the centre of the red cell and displaced the host cell nucleus terminally, were also present. These probably belong to a different species, but the material is insufficient for description.

Morphology (Plate 8, Figs. 10–12)

Slender encapsulated vermicules measured 14–16 μ by 2–2·5 μ, with pale cytoplasm, and particularly small granular nuclei situated near the mid-point (Fig. 12). The capsule appeared to be relatively large and loose, and the vermicules are probably thinner than the above measurements indicate. One free vermicule measured 20 by 1·5 μ, with bluish cytoplasm containing a few pink granules, and a narrow granular nucleus. A few small crescentic forms measuring about 8 by 2 μ were seen (Fig. 10). Larger unencapsulated trophozoites measured 13–14 μ by 3–4 μ, and had blue vacuolated cytoplasm and centrally placed nuclei consisting of widely separated granules or short strands of chromatin (Fig. 11). Normal red cells measured 14–17·5 μ by 8·5–11·5 μ; infected cells 17–21 μ by 10–12 μ; their nuclei were usually displaced laterally and sometimes rather flattened.

This species is distinguished from others by the sparseness of its nuclear material.

HAEMOGREGARINA DARWINIENSIS Lewis

Haemogregarina darwiniensis Lewis, 1913, p. 136.

Host.—Pseudechis australis (Gray).

Distribution.—Northern Territory.

Lewis reported the presence of haemogregarines in several individuals of the common light brown snake taken on the mainland and also on Melville and Bathurst Is. This species was then known as *P. darwiniensis* Macleay, hence the specific name of the parasite.

The parasites varied from long forms, with a centrally disposed nucleus, to blunt spherical forms, with a proportionately much larger nucleus which had lost its reticulated structure. Pigment was aggregated at the rounded extremities of the cell in some of the older parasites. The affected cells were always distorted to some extent and the nucleus was displaced and stained poorly. The illustrations are suggestive of trophozoites rather than intracellular vermicules.

Johnston (1916) considered that *Hg. darwiniensis* might be a synonym of *Hg. pseudechis*. I have not seen any material from the Northern Territory, and, in view of the difficulty in identifying the numerous species of haemogregarines in *Pseudechis* spp. in Queensland, and of the fact that apparently only trophozoites were figured, I would prefer to leave it as unrecognized.

HAEMOGREGARINA DENISONIAE, sp. nov.

Hosts.—*Denisonia pallidiceps* (Günther), *D. signata* (Jan), *Pseudechis porphyriacus* (Shaw), *Notechis scutatus* (Peters).

Distribution.—Mt. Nebo, Fraser I., Innisfail, Qld.

Type.—Slide from *D. pallidiceps* from Innisfail, in the Queensland Museum, Brisbane.

Morphology (Plate 8, Figs. 13–18; Plate 9, Fig. 10)

A moderately heavy infection was present in films made by Miss M. L. Emanuel from a *D. pallidiceps* from Innisfail. Most parasites were encapsulated elongate forms, of medium width, measuring 15–18 μ by 2–3 μ (Fig. 14). The cytoplasm stained pinkish and the nucleus dark red with Giemsa's stain. It was usually elongate and dense but in several specimens the chromatin was distinctly divided into two masses (Fig. 15). In some, a tail was clearly seen tucked up at the narrower end. Very scanty, small, asexual, unencapsulated forms with large nuclei were also seen (Fig. 13). Normal cells measured 15–18·5 μ by 8·5–11 μ; infected cells 17–20 μ by 10–13 μ. They were thus distinctly enlarged, but their nuclei were not distorted and seldom even displaced. Sections of lung and liver were searched, but no schizonts were found.

A very scanty infection was found in a *D. signata* collected by Mr. T. Lawton at Mt. Nebo. The parasites appeared very similar to those described above, except that they were slightly plumper (Fig. 16). Parasites which appeared to be morphologically identical with *Hg. denisoniae* were also present in a *P. porphyriacus* collected on Fraser I. by Mr. D. Mackerras. Long, slender, encapsulated, intracellular, and extracellular vermicules were present (Figs. 17 and 18). Similar parasites were seen in a specimen of *N. scutatus* from Mt. Nebo (Plate 9, Fig. 10).

HAEMOGREGARINA ASPIDOMORPHI, sp. nov.

Hosts.—*Aspidomorphus harriettae* (Krefft), *Notechis scutatus* (Peters), *?Stegonotus plumbeus* (Macleay).

Distribution.—Brisbane, Mt. Nebo, ?Innisfail, Qld.

Type.—Slide from *A. harriettae* from Brisbane, in the Queensland Museum, Brisbane.

Morphology (Plate 9, Figs. 1–7)

Numerous haemogregarines were found in a snake taken near Brisbane by Mr. W. Scott of this Institute. Most were elongate slender forms, each enclosed in a delicate capsule. They measured 14–16 μ by 2–2·5 μ. The cytoplasm stained a very pale pink and was free from granules. The nucleus was rather narrow, appearing reticular or fairly dense according to the heaviness of the stain, and was situated slightly towards one end (Fig. 3). Free vermicules measured 16–20 μ by 2–3 μ with open reticular nuclei (Fig. 4). In some the cytoplasm contained chromatinic granules. There were some slender crescentic bodies, about 10 μ by 1·5 μ. One small trophozoite measured 8 by 3 μ, with a broad reticular nucleus.

Others were larger, 13–14 μ by 2–3 μ, with large, reticular, centrally placed nuclei. The cytoplasm stained pale blue and contained vacuoles (Figs. 1 and 2). The parasite caused only slight elongation of the host cells. Normal red cells measured 16–18·5 μ by 7·5–10·5 μ; infected cells 16–20 μ by 8–10 μ; the nucleus was sometimes slightly elongated but not usually much displaced.

Two kinds of schizonts were found fairly readily in sections of the liver. Young X schizonts were from 20–30 μ in diameter, and showed an undifferentiated central mass, with nuclei arranged peripherally (Fig. 6); older schizonts contained numerous small elongate parasites, each about 7 by 2 μ (Fig. 7). Y schizonts were ovoid bodies, about 20 by 12 μ, with few merozoites, which measured about 14 by 2 μ, and had small, round, dense nuclei (Fig. 5).

This species resembles *Hg. pythonis* in pythons in size and general appearance, but the free vermicules are slightly broader, with more rounded ends. It is also like some specimens seen in *Stegonotus plumbeus* (p. 99). The three forms may prove to be conspecific when more is known about their life histories and host specificity.

Haemogregarines in Other Elapidae

In Notechis scutatus (Plate 9, Figs. 8–15)

At least four distinct species of haemogregarines were found in a tiger snake collected at Mt. Nebo by Mr. T. Lawton.

(1) There were numerous encapsulated parasites, measuring 12·5–14 μ by 2–3 μ, with almost colourless cytoplasm, and medium-sized, reticular, or fairly dense nuclei, which were often wedge-shaped, and which stained reddish with Romanowsky stains (Fig. 8). A slender free vermicule measured 17·5 μ by 2 μ, and had pale homogeneous cytoplasm and a fairly dense elongate nucleus situated nearer the narrower end (Fig. 9). Cells containing these rather short vermicules were slightly enlarged, and their nuclei somewhat displaced. They resemble *Hg. aspidomorphi*, differing only in being a little shorter.

(2) There were numerous encapsulated vermicules, 17–20 μ by 2–2·5 μ, with pale cytoplasm and fairly large, elongate, densely stained nuclei (Fig. 10). They could not be separated morphologically from *Hg. denisoniae*.

(3) A few specimens of a karyolytic species were found. They measured 12·5 by 4 μ, were unstained, and applied closely to the red cell nucleus. The infected cells measured about 29 by 16 μ. These were similar to *Hg. bancrofti* but the infection was too scanty for useful study.

(4) A stout species with a large nucleus was also present in moderate numbers. Trophozoites measured 8–11 μ by 4–6 μ, with particularly large nuclei composed of thick chromatin strands wrapped round the body (Fig. 12). The vermicules were broad, encapsulated, 14–16 μ by 4–5 μ, with large, oblong or wedge-shaped nuclei and pale cytoplasm (Figs. 11 and 13). The nuclei of these organisms stained purple or blue, contrasting with the reddish nuclei of the smallest vermicules. Infected cells were only slightly enlarged. This species cannot be separated morphologically from *Hg. shattocki* in pythons.

X schizonts (Figs. 14 and 15) were found in the liver of this snake. They resembled those seen in a python infected with *Hg. shattocki* (Plate 6, Figs. 10 and 11).

In Pseudechis guttatus

Scanty haemogregarines were found in a slate-bellied black snake from Mt. Nebo. They were rather broad unencapsulated forms with large nuclei. They resembled the asexual forms of *Hg. shattocki* (Plate 6, Fig. 7).

In Acanthophis antarcticus (*Shaw*)

Haemogregarines were found by Scott (1928) in a death adder which died in the Zoological Gardens, London.

In Demansia psammophis reticulata (*Gray*)

Haemogregarines were recorded by Johnston and Cleland (1910) in a spinifex snake from the north-west of Western Australia. They were relatively large stout parasites, measuring 14–16 by 4 μ, with band-like nucleus, and lay lateral to the host cell nucleus. Some were encapsulated. The infected cells were only slightly enlarged and their nuclei were not distorted, merely displaced a little laterally.

KEY TO SPECIES OF HAEMOGREGARINES IN AUSTRALIAN ELAPIDAE*

(Based on the appearance of the intracellular vermicule)

1. Causing marked enlargement of red cell ..... *bancrofti*
   Not causing marked enlargement of red cell 2
2. Short, broad, unstained, usually polar in host cell ..... *australis*
   Well stained, usually lateral in host cell 3
3. Long, rather slender, with elongate dense nucleus ..... *denisoniae*
   Short or of medium length 4
4. Broad forms 5
   Slender forms 6
5. Short to medium forms, fairly broad, with medium-sized, reticular nucleus ... *pseudechis*
   Of medium length, broad, with very large, reticular nucleus ..... *shattocki*
6. Of medium length, with very small, granular, nearly centrally placed nucleus ..... *eidsvoldensis*
   Of medium length, with narrow dense nucleus in posterior half of body ... *aspidomorphi*

* Insufficient data to place *darwiniensis*.

VI. Order HAEMOSPORIDIA

Genus PLASMODIUM Marchiafava & Celli

PLASMODIUM GIGANTEUM Theiler

Plasmodium giganteum Theiler, 1930, p. 493.
Australian host.—*Amphibolurus barbatus* (Cuvier).
Australian distribution.—Wacol, Deception Bay, Eidsvold, S. Qld.

The first studies of the malaria parasites of agamid lizards were made by Wenyon (1909, 1915) in the Sudan. He described *P. agamae* from *Agama colonorum*, a small parasite producing six merozoites. Adler (1924) examined the same species of lizard in Sierra Leone, finding parasites similar to those described

by Wenyon, except that up to 70 merozoites were formed. Theiler (1930) described *P. giganteum* from the same species of lizard from Liberia, differentiating it from *P. agamae* by its larger size, greater number of merozoites, and effect on the red cell, which it caused to become enlarged and stain abnormally. Garnham and Duke (1953) found exoerythrocytic schizonts of *P. agamae* in monocytes in the peripheral blood. Bray (1959) redescribed both species from *A. agama agama* (= *colonorum*), again from Liberia. He found *agamae* with 4–8 merozoites in normocytes, and *giganteum* with 44–96 merozoites in polychromatophil erythroblasts. A few, small, exoerythrocytic schizonts of *agamae* were found in the spleen, one elongate exoerythrocytic schizont of *giganteum* was found in a brain capillary, and a small one of the same species in a macrophage in the liver.

The parasites seen in 3 out of 72 *Amphibolurus barbatus* from various localities in south Queensland agree quite well with those described by Theiler, and have been tentatively assigned to *P. giganteum*. Bray has questioned the statement by Theiler that the host cells become enlarged and stain abnormally, stating that the host cell is "never a mature erythrocyte". This is certainly a point of distinction from the parasites of *Amphibolurus*, which appear to be in mature red cells, but it seems an inadequate reason to establish a new species.

Morphology (Plate 10, Figs. 1–6)

The youngest parasites seen were minute rings about 1·2 μ in diameter, usually placed at one end of a mature red cell, which was not enlarged. With growth of the trophozoite, the host cell became slightly enlarged and its cytoplasm sometimes took a slightly darker stain than normal. Larger rings, with large central vacuole and single well-defined nucleus, measured about 3·4 μ in diameter (Fig. 1). Some were almost triangular in outline. Pigment was not visible in these. Early schizonts, 6–8μ in diameter, had blue cytoplasm with medium-sized grains of black pigment scattered through it, and two or more nuclei. A slightly larger schizont, 9 μ in diameter, contained 15 nuclei and an aggregation of pigment granules near its centre. It occupied one end of the red cell and displaced the nucleus slightly. Larger schizonts became ovoid or club-shaped (Fig. 2), some enveloping the nucleus and almost filling one side and both ends of the host cell. One mature schizont had formed 22 and another, the largest seen, 49 merozoites (Fig. 3).

The gametocytes were large, oval or sausage-shaped parasites. The macrogametocytes (Figs. 4 and 5) stained blue and the microgametocytes (Fig. 6) were almost colourless. The nuclei were sometimes dispersed in several masses. The pigment varied in amount and texture from scanty fine granules to relatively abundant, round, black ones. Normal red cells measured 11·5–17 μ by 7–9·5 μ; cells containing gametocytes were 14·5–18 μ by 8–11 μ; those containing schizonts were 15–20 μ by 7–12 μ; the nucleus was usually displaced laterally but was not distorted.

Experimental

The first infected lizard obtained showed a moderate infection with large parasites, which were probably all gametocytes. It was kept in captivity for 4 weeks and its blood examined on 14 occasions. No changes in the blood picture

occurred and schizonts were never seen, but exflagellation was observed once. Blood from this lizard was inoculated intraperitoneally into three other lizards of the same species, but no parasites were found subsequently in any of the recipients, which were examined from 10 to 14 times during the ensuing 5 weeks. The second infected lizard showed a very scanty infection of what were probably gametocytes. Splenectomy was performed and the blood examined on 14 occasions during the ensuing 38 days. A few parasites were found on six occasions but their density remained low. These animals were lost prematurely in an epizootic of salmonellosis.

The third infection was found some 5 years later in a blood film and carcass of a lizard kindly submitted by Messrs. A. Drayden and H. Ronken of the Anatomy Department, University of Queensland. The blood showed fairly numerous young trophozoites, scanty schizonts, and an occasional gametocyte. No exoerythrocytic schizonts were found in sections of liver, spleen, or lung of this lizard.

Plasmodium egerniae, sp. nov.

Host.—Egernia major major (Gray).

Distribution.—Innisfail, N. Qld.

Type.—Slide from *E. m. major* from Innisfail, in the Queensland Museum, Brisbane.

Films were received from three infected lizards taken in the rain-forest by Miss M. L. Emanuel. In the first, there were very scanty trophozites and one early schizont was found. In the second, there were moderately numerous young trophozoites, very scanty schizonts (only one being found), and very scanty gametocytes. In the third, only scanty gametocytes were present.

Morphology (Plate 10, Figs. 7–12)

The youngest forms seen were tiny rings about 1–2 μ in diameter (Fig. 7). As they grew they tended to become elongate or more or less triangular in outline, with an irregularly shaped nucleus, and usually a single well-defined spot of pigment. They were in mature red cells. One early schizont measured 8 by 4 μ, with the chromatin arranged in four masses around the periphery and with numerous fine grains of pigment aggregated at one end. The parasite lay at one end of the red cell, which was not enlarged nor distorted (Fig. 8). One nearly mature schizont filled the red cell and displaced its nucleus to the edge. There were between 40 and 50 discrete masses of chromatin but the division into merozoites had not been completed (Fig. 9).

The gametocytes were elongate ovoid bodies, filling the red cells, and pushing the enlarged and distorted host cell nucleus to the periphery. Microgametocytes stained faintly, the nucleus was usually a single mass, and the pigment polar in distribution (Fig. 12). Macrogametocytes stained blue, with some vacuoles and scattered grains of pigment, and the nucleus was small (Figs. 10 and 11). Normal red cells measured 14·5–17 μ by 7–9 μ; cells containing gametocytes measured

19–22 μ by 9–12 μ, and their nuclei were drawn out into band-like or wedge-shaped objects, measuring up to 20 μ in length by 1–3 μ in width, squeezed between the red cell envelope and the parasite.

Relationships

Seven species of *Plasmodium* are known from skinks in various parts of the world. The young trophozoites of all are very similar but the species may be distinguished from one another by the characters listed in Table 2.

Genus HAEMOCYSTIDIUM Castellani & Willey

Castellani and Willey erected this genus in 1904 for a pigmented parasite of a tree gecko, *Hemidactylus leschenaultii*, from Ceylon. They suggested the name on account of the turgid bladder-like appearance of some of the parasites. Later workers (Shortt 1922*a*; Wenyon 1926) considered *Haemocystidium* to be a synonym of *Haemoproteus* Kruse.

However, the pigmented parasites seen in Australian geckos and tortoises do not resemble the *Haemoproteus* of birds at all closely, being usually spherical or ovoid, and only occasionally halteridium-like. The frequent occurrence of more than one nucleus and the marked discrepancy in size of the male and female gametocytes in the parasites of the geckos are not typical of the genus *Haemoproteus*. Nothing at all is known of the life history of any of these parasites of reptiles, and it therefore seems reasonable to keep them apart from the well-defined group of avian parasites belonging to the genus *Haemoproteus*, which, moreover, is already overloaded with species, and is likely to become even more so.

It is proposed to use the generic name *Haemocystidium* Castellani & Willey for the pigmented parasites of the red cells of reptiles, which are usually ovoid or spherical, and in which erythrocytic schizogony has not been observed.

Haemocystidium chelodinae Johnston & Cleland

Haemocystidium (*Plasmodium*) *chelodinae* Johnston and Cleland, 1909, p. 101. Type slide from *Chelodina longicollis* from Sydney, in the Australian Museum, Sydney.
Haemoproteus chelodinae (Johnston and Cleland, 1909), Shortt, 1922*a*, p. 820.

Hosts.—*Chelodina longicollis* (Shaw), *C. oblonga* Gray, *Emydura macquarii* (Gray), *E. latisternum* (Gray), *E. krefftii* (Gray), *Elseya dentata* (Gray).

Distribution.—Sydney; Brisbane, Mt. Nebo, Eidsvold, S. Qld.; Perth.

Johnston and Cleland described this parasite from 1 out of 2 *C. longicollis* obtained near Sydney in 1909. The parasites present were thought to be gametocytes. They were usually oval or rounded, measuring from 4 by 3 μ up to 12 by 7 μ, but kidney-shaped forms were seen occasionally. The cytoplasm was pale in some and deeply stained in others; one or more vacuoles were present in both forms. Pigment was sometimes granular, sometimes in short rods; in some it was dispersed throughout the organism, and in others it was grouped in little masses, often towards the centre. The parasite occupied one end of the cell, which was not distorted, nor was its nucleus displaced.

TABLE 2
SPECIES OF PLASMODIUM RECORDED FROM SCINCIDAE

Species	Host	Locality	No. of Merozoites	Size and Shape of Gametocytes	Effect of Gametocytes upon Red Cell
P. mabuiae Wenyon, 1909	*Mabuia quinquetaeniata*	Sudan	6	Small, ovoid	Nil
P. minasense Carini & Rudolph, 1912	*M. agilis*	Brazil	4	Small, spherical, ovoid, or elongate	Nil
P. maculilabre Schwetz, 1931	*M. maculilabris*	Belgian Congo	20	Large	Considerable hypertrophy, nucleus displaced
P. pitmani Hoare, 1932	*M. striata* *M. maculilabris*	Uganda, Kenya	18–20	Medium, oval or elongate	Nil
P. lacertiliae Thompson & Hart, 1946	*Leiolopisma fusca*	Goodenough I.	10	Irregularly oval, indented by red cell nucleus	Slight hypertrophy, nucleus displaced
P. lygosomae Laird, 1951	*Lygosoma moco*	New Zealand	4	Reniform or elongate	Nil
P. egerniae, sp. nov.	*Egernia major major*	Queensland	40	Large, irregularly ovoid	Enlargement of cell and marked distortion of nucleus

Johnston and Cleland (1910) recorded these parasites in *E. krefftii* from Queensland, but in 1912 Johnston corrected the host identification to *E. macquarii*. Johnston and Cleland (1912) reported finding a few parasites in *C. oblonga* from Perth, and amplified their previous description, giving the average size as 11 by 8 μ.

Morphology (Plate 11, Figs. 1–3)

Scanty infections have been seen in *E. latisternum* from Mt. Nebo, in *E. krefftii* and *Elseya dentata* from Eidsvold, and in *C. longicollis* from Brisbane. The parasites agreed well with the original description. Round forms, 11 μ by 9 μ, with blue cytoplasm, contained 1–3 sharply defined vacuoles and a small elongate nucleus, usually situated peripherally. These were regarded as macrogametocytes (Fig. 2). The pigment appeared as fairly abundant rods or granules usually scattered through the cytoplasm but sometimes aggregated into irregular masses. Smaller parasites, about 8 μ by 7 μ, which stained pink and had a rather diffuse nucleus, were regarded as microgametocytes (Fig. 3). No vacuoles were seen in them but only very few were found. The pigment was usually in clumps. Forms, which were often as large as the macrogametocytes but which had pinkish blue vacuolated cytoplasm, were regarded as immature macrogametocytes (Fig. 1).

Only slight enlargement of the infected cells occurred. Normal red cells of *E. latisternum* measured 19–25 μ by 10–14·5 μ; infected cells 20–26 μ by 11–15 μ.

Relationships

Johnston and Cleland considered their form to be distinct from *Haemocystidium metchnikovi* (Simond, 1901) in the Indian river tortoise, *Trionyx indicus*, because it was larger and had larger and more abundant pigment granules. Wenyon (1926) placed these parasites in the genus *Haemoproteus*, and thought that probably all species described from tortoises belonged to *metchnikovi*. Hewitt (1940) recorded the parasites of a North American terrapin, *Pseudemys elegans*, as *Haemoproteus metchnikovi*. Simond's original illustrations (Simond 1901) are rather difficult to interpret, but the parasites seen in *E. latisternum* (Figs. 1–3) seem to differ from those figured by Hewitt, which were smaller, often reniform, with a nearly central nucleus. The two species are evidently closely related, but it is proposed to retain the name *chelodinae* for the parasites of Australian tortoises until a comparison can be made with material from India.

HAEMOCYSTIDIUM SIMONDI Castellani & Willey

Haemocystidium simondi Castellani and Willey, 1904, p. 84.
Haemoproteus simondi (Castellani and Willey), Wenyon, 1926, p. 902.

Australian hosts.—*Phyllurus platurus* (Shaw), *Heteronota binoei* Gray, *Oedura tyroni* de Vis, *Gehyra variegata australis* Gray.

Australian distribution.—Sydney, Glen Davis, N.S.W.; Eidsvold, Mornington I., Qld.

Castellani and Willey (1904) described this species from *Hemidactylus leschenaultii* from Ceylon. The earliest stage was a small, rather irregular, amoeboid

body, with a zone of pigment granules across the centre. As the parasites grew, they tended to fill the red cell and to displace the nucleus to one end. Male gametocytes stained a pale blue, with numerous small pigment granules around the periphery. Female gametocytes stained dark blue and the pigment granules were slightly larger than those of the male. Vacuoles were noted in the cytoplasm of the females only. The authors did not describe the nucleus.

Johnston (1912) recorded that Dr. J. B. Cleland and he had found *Haemocystidium* sp. in a film from *O. tryoni* sent to them by Dr. T. L. Bancroft from Eidsvold, and I have seen parasites in 2 out of 14 of this species from the same locality. Parasites were numerous in one, with many multiply infected cells, but were scanty in the other infection.

Similar pigmented parasites were seen in 17 out of 69 *P. platurus*, from various localities on the Hawkesbury sandstone immediately north of Sydney, and the Rev. R. Palmer has kindly sent me a photomicrograph of a typical parasite in a blood film from the same species of gecko from Glen Davis. Parasites were abundant in six of the lizards studied, fairly numerous in four, and scanty in the remainder.

A rather scanty infection of pigmented parasites was seen in 1 out of 12 *G. v. australis* examined at Mornington I. No parasites of this nature were found in 26 specimens examined at Eidsvold, nor in 2 at Low I., Qld. Three out of 7 *H. binoei* obtained on Mornington I. were infected, one very heavily and the others moderately so.

Morphology (Plate 11, Figs. 4–12)

Two forms were present in stained films, one staining a dark blue with Romanowsky stains, and the other very light blue or pale pink. The dark blue forms, which were regarded as macrogametocytes, varied from small round or oval bodies, measuring 6–7 μ by 4–6 μ, occupying one end of the red cell, up to large, ovoid, irregularly shaped, or halteridium-like forms occupying most of the red cell. Some of these measured 22 μ in length by 4–6 μ in breadth. There was abundant moderately coarse pigment dispersed throughout the body. The most remarkable aspect was the failure of the nucleus to take the stain in many parasites. A well-defined nucleus surrounded by a clear halo was, however, present in some, being more readily found in some lizards than in others (Fig. 11). Even in smears in which many parasites became extracellular, many large, round or irregularly shaped, blue parasites appeared to be devoid of a nucleus. Small vacuoles were seen in some (Fig. 8).

The pale forms, which were regarded as microgametocytes, measured 5–8 μ by 6 μ, and possessed fairly well-defined nuclei (Figs. 4, 6, 7, 9). Usually one was present, but sometimes there were two, or even three, small, densely stained chromatin masses (Fig. 9). The pigment granules were finer than those in the macrogametocytes (Fig. 8).

In films from heavily infected lizards (Figs. 4, 6, 9, 12), many cells contained more than one parasite. In a specimen of *H. binoei*, in which about 35% of the red cells were infected, 36% of the infected cells had a single parasite, 44% had

2, and 20% had from 3–6 parasites. Male gametocytes outnumbered the females by 6 : 1 in this film, and their maturity was shown by the occurrence of very numerous exflagellations in a specimen of tail blood, in which every microscopic field seethed with microgametes. In other lizards, the sex ratio was nearly 1, and sometimes more females were present than males.

In some heavily infected *P. platurus*, the parasites appeared as multinucleate masses surrounding the host cell nucleus. However, in many films, particularly in those fixed in Schaudinn's fixative and stained with Delafield's haematoxylin, the large mass was clearly seen to be composed of a number of small parasites closely approximated. The individual parasites were frequently unlike in their staining reactions, which made it more probable that the aggregation of parasites was due to multiple infection of a red cell by young gametocytes, and not to any form of division occurring within the cell. As many as 10 small parasites have been seen liberated by a burst red cell (Fig. 12).

The parasites seen in *G. v. australis* were rather uniformly ovoid or spherical, and no large halteridium forms were found. Many parasites had two small dense nuclei and coarse pigment (Fig. 10).

Normal cells varied a good deal in size. On the whole, the parasites caused surprisingly little enlargement of the infected cells, but cells containing macrogametocytes and multiple infections were broader than normal. Measurements (μ) of normal and infected cells are set out below:

Species	Normal Red Cells	Infected Red Cells
P. platurus	18 –21 by 10–13	18–23 by 12 –15
H. binoei	14 –22 by 8–13	15–23 by 8 –15
O. tryoni	17 –20 by 9–11	16–20·5 by 9 –13
G. v. australis	16·5–19 by 8–10	17–19 by 8·5–12

Sections of liver, lung, heart, spleen, pancreas, intestine, brain, muscle, and skin of a heavily infected *H. binoei* were examined, but not schizonts were found.

No tiny forms, such as are seen in *Plasmodium* infections, and nothing resembling a *Plasmodium* schizont were seen in any of the films examined. A characteristic feature was the discrepancy in size between the microgametocytes, which seldom exceeded 7–8 μ in diameter, and did not assume a halteridium shape, and the macrogametocytes, which sometimes filled the red cell, and curled round the ends of the nucleus like a very plump *Haemoproteus* (Fig. 11). Vacuolation of the cytoplasm, which was characteristic of the macrogametocytes of *Hc. chelodinae*, was not marked in the parasites of geckos. Very few vacuoles were seen, and sometimes only one small one was present. Some microgametocytes contained irregularly shaped clear spaces, suggestive of artifacts rather than vacuoles.

Experimental

The occurrence of exflagellation in *H. binoei* has already been mentioned and it has also been observed in *P. platurus* and *O. tryoni*, at least five microgametes being formed. In wet preparations many macrogametocytes became extracellular, rounding up, with dancing pigment.

Eleven *Culex fatigans,* which fed on *H. binoei* infected with *Hg. heteronotae* and *Hc. simondi,* were dissected at intervals from 12 hr to 22 days but no developmental stages were seen.

Relationships

Several species of *Haemocystidium* or *Haemoproteus* have been described from geckos in other parts of the world. Of these, *Haemoproteus phyllodactyli* Shortt, 1922, in the Persian gecko, *Phyllodactylus elisae,* seems to be quite distinctive in having elongate, halteridium-like male, as well as female, gametocytes. *Haemocystidium kopki* de Mello, 1916, from the Indian gecko, *Hemidactylus brookei,* also seems to be distinct in having males with vacuoles containing pigment and females with uniform cytoplasm. *Haemocystidium tarentolae* described by Parrot (1927) from the Algerian gecko, *Tarentola mauritanica,* and *Haemoproteus tarentolae,* described by Riding (1930) from the Sudanese gecko, *Tarentola annularis,* seem to have differences in nuclear structure, but are probably both close to *Hc. simondi.* The forms in Australian geckos agree best with *Hc. simondi,* although it is possible that there may be more than one species among them. De Mello (1934) found scattered merozoites and small schizonts in the lung, but no one else has reported finding any stages of multiplication.

VII. Parasites of Uncertain Classification

Genus PIRHEMOCYTON Chatton & Blanc

Pirhemocyton tarentolae Chatton & Blanc

Pirhemocyton tarentolae Chatton and Blanc, 1914, p. 496.

Australian hosts.—Phyllurus platurus (Shaw), *Gehyra variegata australis* Gray.

Australian distribution.—Sydney, Glen Davis, N.S.W.; Eidsvold, Qld.

Chatton and Blanc (1914, 1916) described some peculiar organisms in the red cells of a gecko, *Tarentola mauritanica,* from North Africa. The smallest forms resembled anaplasmata, appearing as red dots about 1 μ in diameter. Larger individuals measured 3–4 μ in diameter and consisted of a central chromatin dot surrounded by clear cytoplasm. Associated with each parasite was a large globular body, which appeared refractile in the fresh state and as a clear space in stained films. This body was separate from the parasite proper, lying in another part of the red cell. Blanc and Ascione (1959) recognized 4 species in lizards, 2 in Lacertidae, 1 in geckos, and 1 in a chameleon. The characters used to separate species are not convincing, and all the forms seen in Australian geckos have been provisionally allotted to *Pr. tarentolae.*

Bearup (1951) recorded the presence of these organisms, along with haemogregarines, in blood films of *P. platurus,* obtained by the Rev. R. Palmer at Glen Davis. It was a common parasite of the same species of gecko from various localities immediately north of Sydney, being present in 18 out of 69 individuals examined. The infections varied from very scanty to extremely abundant; it was not unusual to find about half the red cells infected, and some infections were heavier.

Morphology (Plate 12, Figs. 1–4)

The parasites in *P. platurus* agreed well with the original description, except that a zone of clear cytoplasm could not be detected around the chromatin body, which was usually in close contact with the globular unstained body (Fig. 2), although they were sometimes separate (Fig. 1). The globules varied from 2–12 μ in diameter and the chromatin bodies were usually about 1–2 μ in diameter, occasionally a little larger. In many slides parasites of all sizes were present together, but occasionally the small forms predominated. Double infection of a red cell was only rarely seen. Infected cells were slightly enlarged, mainly in the transverse axis, appearing a little plumper than normal, and their nuclei were sometimes displaced.

A heavy infection with similar organisms was seen in 1 out of 26 *G. v. australis* from Eidsvold. The parasite differed in some respects from those of *P. platurus*. The chromatin bodies were often considerably larger, sometimes nearly as large as the associated globule. This was particularly noticeable when the parasite was developing in an immature red cell (Fig. 4). The unstained globules were usually smaller and were frequently completely dissociated from the chromatin bodies (Fig. 3), while chromatin bodies without an unstained globule were present in many cells (Fig. 4). Nothing at all is known of the life history of these parasites.

Pirhemocyton sp.

A scanty infection was present in a film from a carpet snake, *Morelia spilotes variegata*, which was given to me by Professor J. F. A. Sprent. The parasites were smaller than those in the geckos, but presented no diagnostic features.

VIII. Acknowledgments

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Explanation of Plates 1–12

All photomicrographs were taken at the same magnification with a Leitz Panphot microscope, using a × 8 periplan eyepiece and a 2-mm apochromatic oil-immersion objective, with the exception of Plate 6, Figure 14, which was taken with an 8-mm objective

Plate 1

Trypanosomes in tortoises and lizards

Fig. 1.—*Trypanosoma chelodina* in *Chelodina longicollis.*

Fig. 2.—*T. chelodina* in *Emydura krefftii.*

Figs. 3 and 4.—*T. phylluri* in *Phyllurus platurus.*

Figs. 5 and 6.—*T. egerniae* in *Egernia striolata.*

Fig. 7.—*T. egerniae in E. cunninghami.*

Plate 2

Haemogregarines in tortoises and lizards

Figs. 1–6.—*Haemogregarina clelandi* in tortoises: 1, young trophozoites in *Chelodina longicollis*; 2 and 3, schizonts in *C. longicollis*; 4, encapsulated vermicule in *Emydura krefftii*; 5, encapsulated vermicule in *C. longicollis* showing tail; 6, encapsulated and free vermicules in *E. krefftii.*

Fig. 7.—*Haemogregarina* sp. in *Phyllurus platurus.*

Figs. 8 and 9.—*Haemogregarina* sp., encapsulated and free vermicules in *Gehyra variegata australis.*

Figs. 10–13.—*Hg. palmeri* in *Physignathus lesueurii*: 10 and 11, trophozoites; 12 and 13, encapsulated and free vermicules.

Figs. 14–17.—*Hg. tiliquae* in *Tiliqua scincoides*: 14 and 15, trophozoites; 16 and 17, encapsulated and free vermicules.

Plate 3

Haemogregarina heteronotae in the gecko, *Heteronota binoei*

Fig. 1.—Young trophozoite.

Fig. 2.—Large trophozoite.

Figs. 3 and 4.—Intracellular vermicules.

Fig. 5.—Free vermicule.

Figs. 6 and 7.—Small cysts in lung: *c.w.*, cyst wall; *h.n.*, host cell nucleus; *p.n.*, parasite nucleus; *v.*, vacuole containing parasite.

Figs. 8–11.—X schizonts in lung: 8, early stage with peripherally arranged nuclei; 9, more advanced stage on left, and edge of mature schizont on right showing four merozoites; 10 and 11, consecutive sections of a mature schizont showing arrangement of merozoites.

Plate 4

Haemogregarines in skinks

Figs. 1–3.—*Haemogregarina hinuliae* in *Sphenomorphus quoyii*: 1 and 2, encapsulated vermicules; 3, free vermicule

Figs. 4, 4*a*, and 5.—*Hg. taeniolati* in *Sphenomorphus taeniolatus*: 4, three encapsulated and one free vermicule, an empty capsule on the right, from *S. taeniolatus* No. 13; 4*a*, large free vermicule showing numerous granules, from *S. taeniolatus* No. 13; 5, trophozoite from *S. taeniolatus* No. 10.

Figs. 6–9.—*Hg. cunninghami* in *Egernia cunninghami*: 6 and 7, trophozoites; 8 and 9, encapsulated and free vermicules.

Fig. 10.—*Haemogregarina* sp. in *Egernia striolata.*

Fig. 11.—*Haemogregarina* sp. in *E. cunninghami.*

Figs. 12 and 13.—*Hg. egerniae* in *E. cunninghami*: 12, trophozoite; 13, unstained vermicule.

Figs. 14 and 15.—*Hg. obscura* in *E. cunninghami.*

Plate 5

Haemogregarines in varanids

Fig. 1.—*Haemogregarina varanicola* in *Varanus varius varius* (type slide).

Figs. 2 and 3.—*Hg. varanicola,* encapsulated and free vermicules in *V. tristis orientalis.*

Figs. 4 and 5.—*Hg. gouldii* in *V. gouldii* (type slide).

Figs. 6 and 7.—*Hg. breinli,* encapsulated and free vermicules in *V. t. orientalis.*

Figs. 8 and 9.—Trophozoites in *V. t. orientalis,* possibly *Hg. breinli.*

Figs. 10–12.—*Hg. gilruthi,* encapsulated and free vermicules in *V. t. orientalis.*

Fig. 13.—Trophozoite in *V. t. orientalis,* possibly *Hg. gilruthi.*

Figs. 14 and 15.—*Hg. johnstoni,* trophozoites in *V. v. varius* No. 6.

Fig. 16.—*Hg. johnstoni,* encapsulated vermicule in *V. v. varius* No. 8.

Fig. 17.—X schizont in liver of *V. v. varius* No. 6.

Fig. 18.—Y schizont in liver of *V. v. varius* No. 6.

Plate 6

Haemogregarines in pythons. Figures 1–13 at same magnification as Figure 15

Figs. 1–4.—*Hg. pythonis*: 1, 2, and 3, encapsulated and free vermicules in *Morelia spilotes variegata* No. 1; 4, encapsulated vermicule in *Chondropython viridis.*

Figs. 5 and 6.—*Hg. pococki* in *M. s. variegata* No. 5: 5, encapsulated vermicule; 6, trophozoite.

Figs. 7–11.—*Hg. shattocki* in *M. s. variegata* No. 4: 7 and 9, trophozoites; 8, encapsulated vermicule; 10 and 11, X schizonts in liver.

Figs. 12 and 13.—*Hg. amethystina* in *M. s. variegata* No. 7.

Figs. 14 and 15.—Y schizonts of *Hg. shattocki* in liver of *M. s. variegata* No. 4: 14, several Y schizonts associated with a clump of pigment-laden macrophages; 15, high-power view of one Y schizont.

Plate 7

Haemogregarines in colubrid snakes

Figs. 1–6.—*Haemogregarina dendrophidis* in *Dendrophis punctulatus*: 1–3, trophozoites in *D. punctulatus* No. 1; 4, crescentic trophozoite in *D. punctulatus* No. 4; 5 and 6, encapsulated and free vermicules in *D. punctulatus* No. 4.

Figs. 7–12.—*Hg. mirabilis* in *Natrix mairii*: 7 and 8, trophozoites; 9 and 10, encapsulated intracellular and extracellular vermicules; 11, X schizont in lung; 12, Y schizont in lung.

Figs. 13–16.—*Hg. stegonoti* in *Stegonotus plumbeus*: 13 and 14, trophozoites; 15, encapsulated vermicule; 16, free vermicule.

Figs. 17 and 18.—*Hg. ?aspidomorphi* in *Stegonotus plumbeus;* 17, crescentic trophozoite; 18, encapsulated vermicule.

Plate 8

Haemogregarines in colubrid and elapid snakes

Figs. 1–4.—Haemogregarines in *Boiga fusca*: 1 and 2, *Hg. boigae*, encapsulated vermicules, *e.*, eosinophilic cap, *g.*, grey globule; 3, *Haemogregarina* sp., encapsulated vermicule; 4, Y schizont in liver.

Figs. 5 and 6.—*Hg. bancrofti* in *Pseudechis guttatus*.

Figs. 7 and 8.—*Hg. australis* in *Pseudechis australis* No. 2: 7, trophozoite; 8, unstained, encapsulated form.

Fig. 9.—*Hg. pseudechis* in *P. australis* No. 2.

Figs. 10–12.—*Hg. eidsvoldensis* in *P. australis* No. 1: 10, crescentic trophozoite; 11, large trophozoite; 12, encapsulated vermicule.

Figs. 13–18.—*Hg. denisoniae* in various snakes: 13, trophozoite in *Denisonia pallidiceps*; 14 and 15, encapsulated vermicules in *D. pallidiceps*; 16, encapsulated vermicule in *Denisonia signata;* 17 and 18, encapsulated intra- and extra-cellular vermicules in *Pseudechis porphyriacus*.

Plate 9

Haemogregarines in elapid snakes

Figs. 1-7.—*Haemogregarina aspidomorphi* in *Aspidomorphus harriettae*: 1 and 2, trophozoites; 3 and 4, encapsulated and free vermicules; 5, Y schizont in liver; 6, early stage X schizont in liver; 7, mature X schizont in liver.

Figs. 8–15.—Haemogregarines in a *Notechis scutatus*: 8 and 9, encapsulated and free vermicules of *Hg. aspidomorphi*; 10, encapsulated vermicule of *Hg. denisoniae*; 11, double infection with different species; 12, small trophozoite, possibly *Hg. shattocki*; 13, encapsulated vermicule of *Hg. shattocki*; 14 and 15, X schizonts, possibly of *Hg. shattocki,* in the liver.

Plate 10

Plasmodium spp. in lizards

Figs. 1–6.—*Plasmodium giganteum* in *Amphibolurus barbatus*: 1, young trophozoites in *A. barbatus* No. 68; 2, nearly mature schizont in No. 68; 3, mature schizont in No. 68; 4 and 5, macrogametocytes in *A. barbatus* No. 10; 6, microgametocyte in No. 10.

Figs. 7–12.—*P. egerniae* in *Egernia major major*: 7, young trophozoite; 8, early schizont with 4 nuclei; 9, nearly mature schizont; 10 and 11, macrogametocytes; 12, microgametocyte.

Plate 11

Haemocystidium spp. in tortoises and geckos

Figs. 1–3.—*Haemocystidium chelodinae* in *Emydura latisternum*: 1, immature macrogametocyte; 2, mature macrogametocyte; 3, mature microgametocyte; *n*, nucleus.

Figs. 4–8.—*Hc. simondi* in *Heteronota binoei*: 4, one free and three intracellular microgametocytes above, one macrogametocyte below; 5, large macrogametocyte, showing paler centre, but without definite nucleus; 6, five microgametocytes; 7, one intracellular and one free microgametocyte; 8, free macrogametocyte above, showing vacuole and coarse pigment, but no nucleus, microgametocyte with faintly stained nucleus (*n*) below.

Fig. 9.—*Hc. simondi,* binucleate macrogametocyte to left, and trinucleate microgametocyte to right, in *Phyllurus platurus* (pigment not visible).

Fig. 10.—*Hc. simondi,* binucleate macrogametocyte in *Gehyra variegata australis*.

Fig. 11.—*Hc. simondi*, macrogametocyte with definite nucleus in *Oedura tryoni*; *n*, nucleus.

Fig. 12.—*Hc. simondi*, microgametocytes liberated from burst red cell in *Phyllurus platurus* (film fixed in Schaudinn's fixative, and stained with Delafield's haematoxylin).

Plate 12

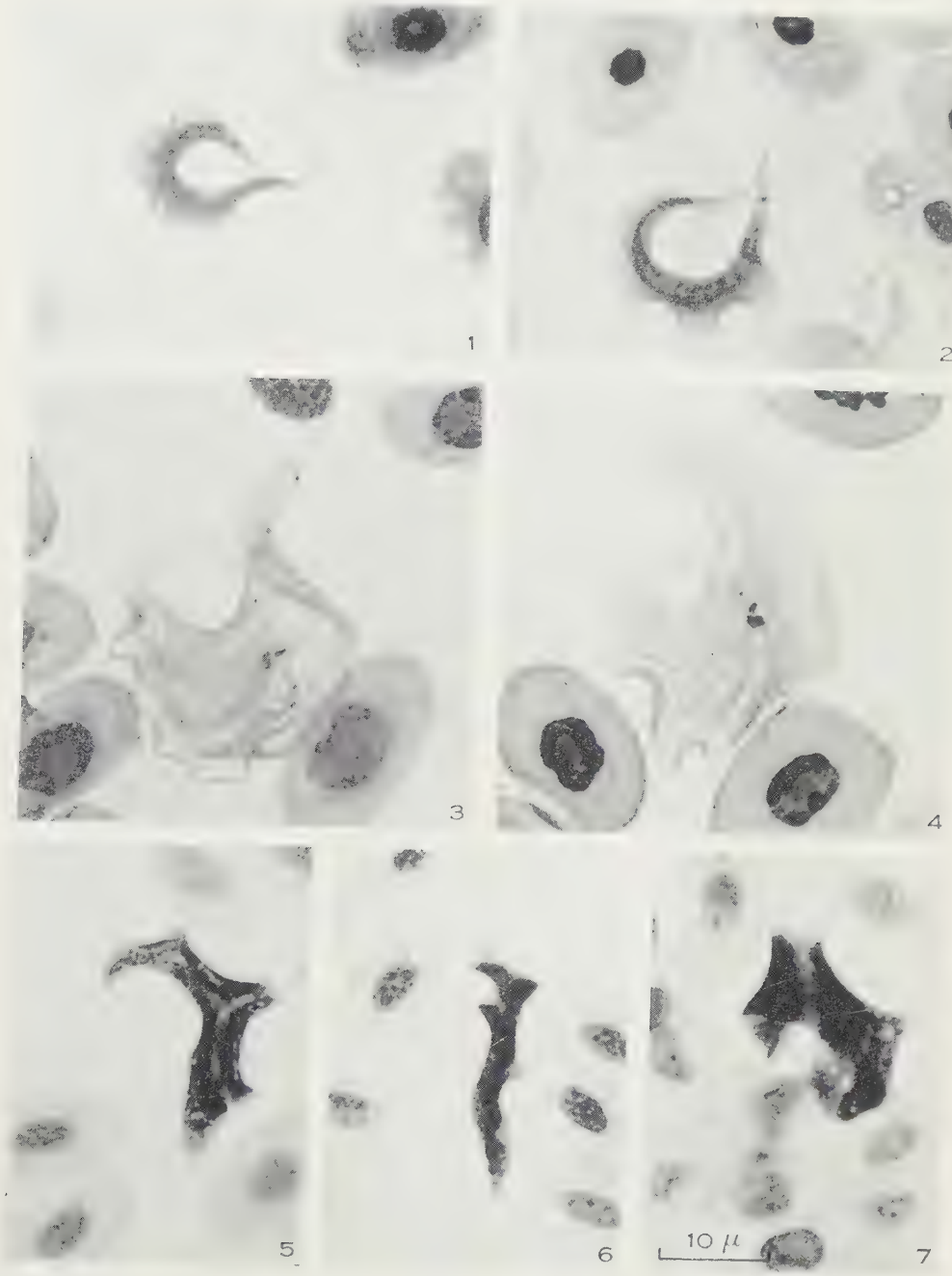
Pirhemocyton in geckos

Figs. 1 and 2.—*Pirhemocyton tarentolae* in *Phyllurus platurus*.

Figs. 3 and 4.—*Pr. tarentolae* in *Gehyra variegata australis*.

Index of Generic and Specific Names

THE HAEMATOZOA OF AUSTRALIAN REPTILES



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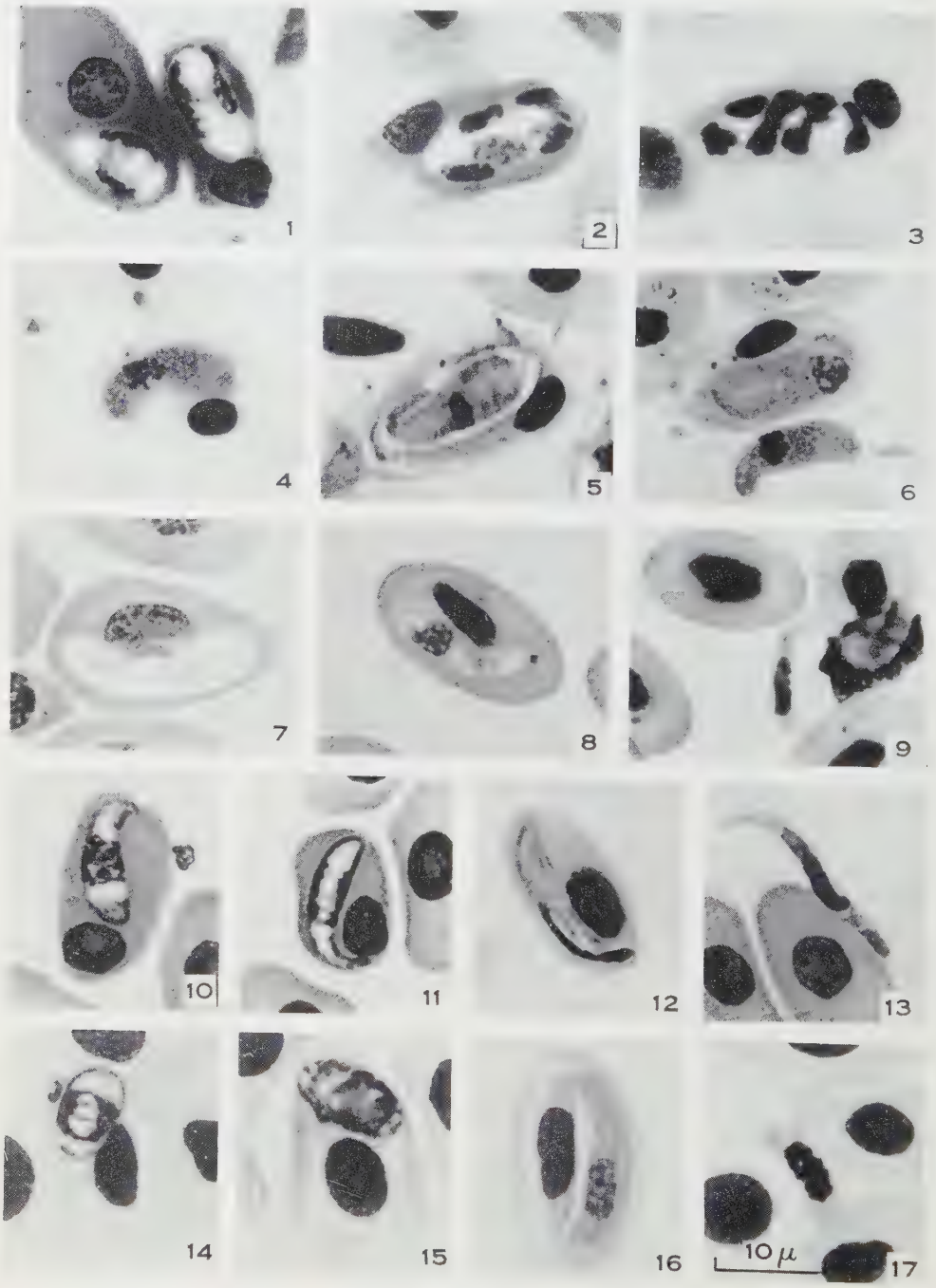

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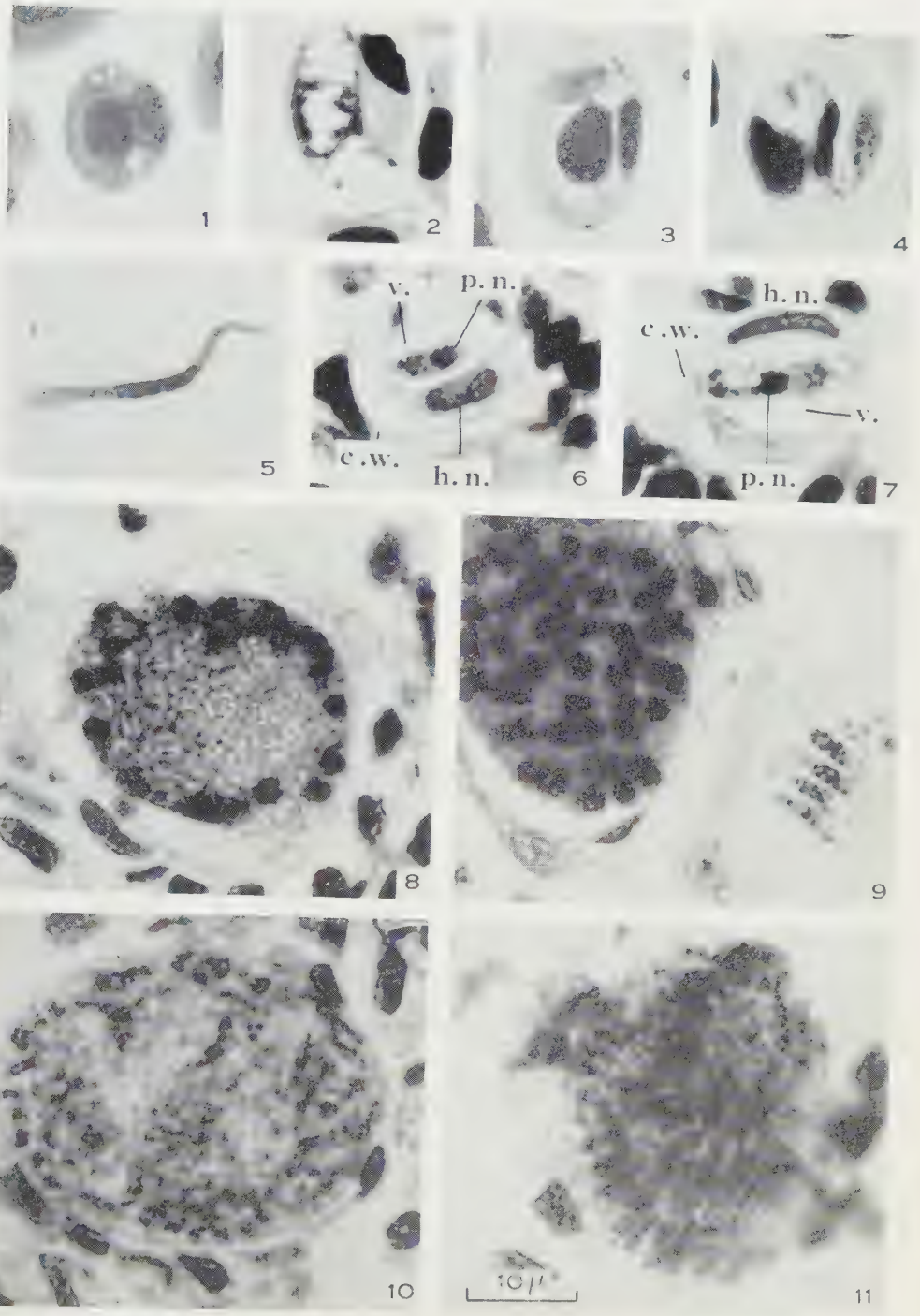

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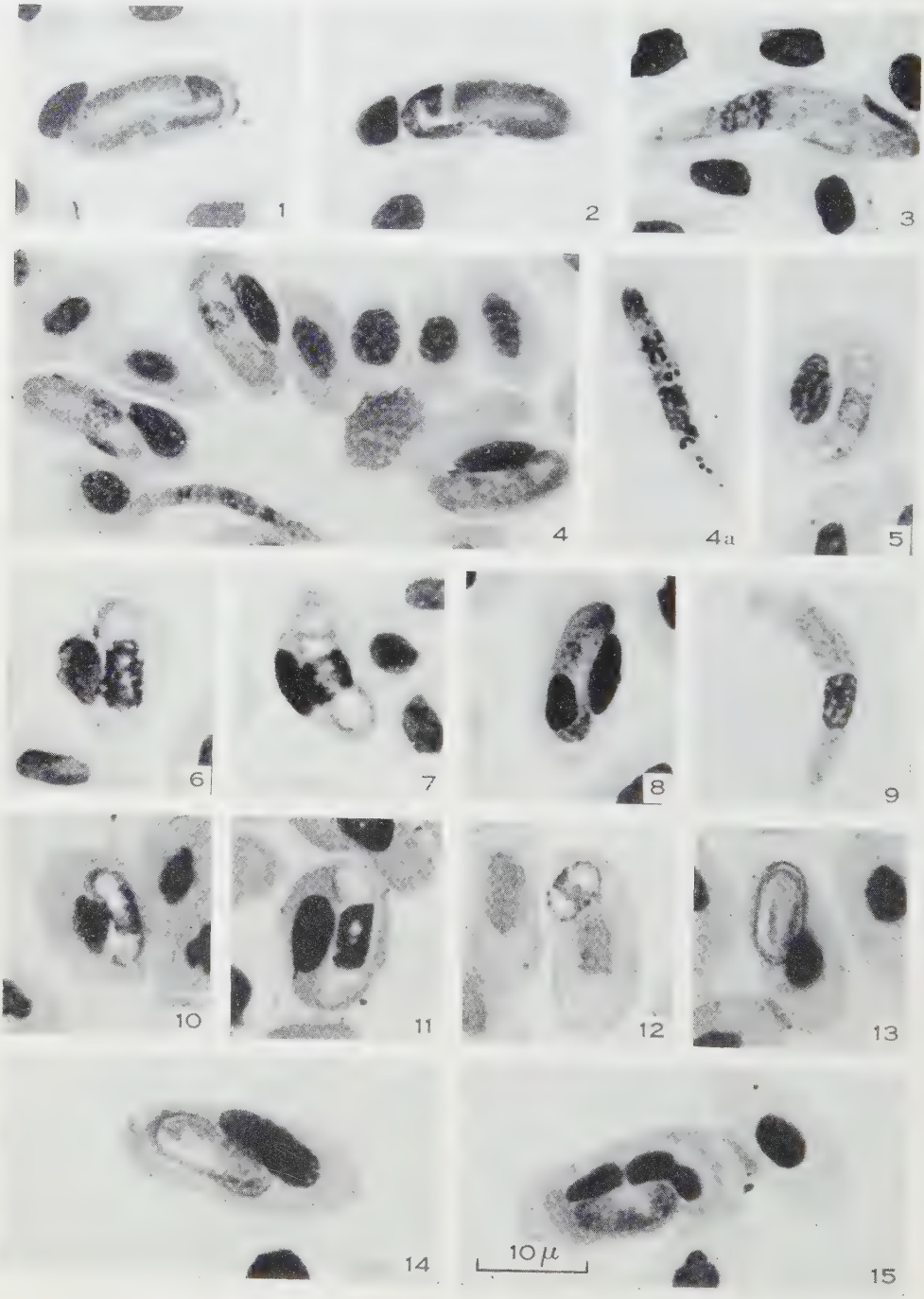

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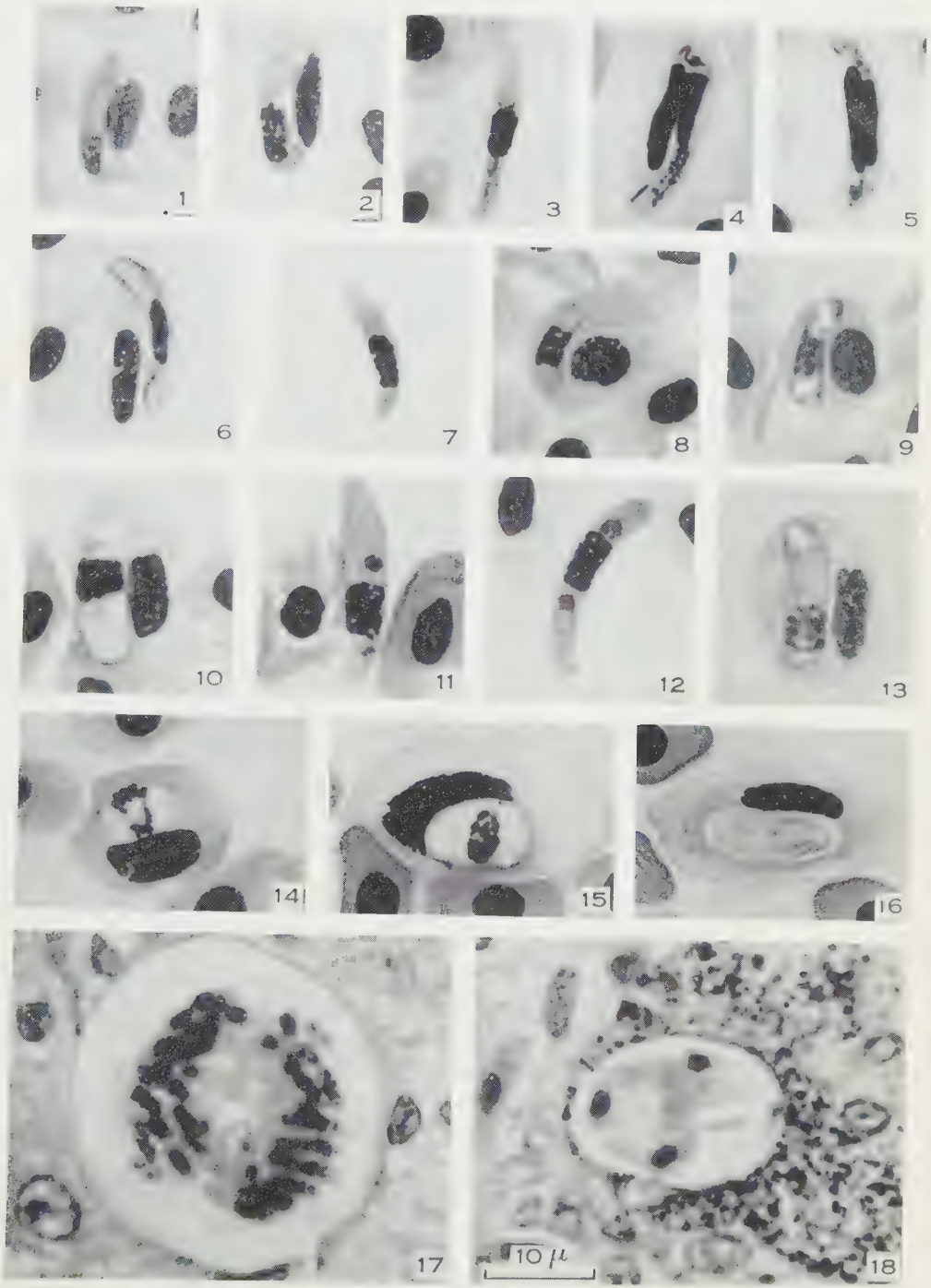

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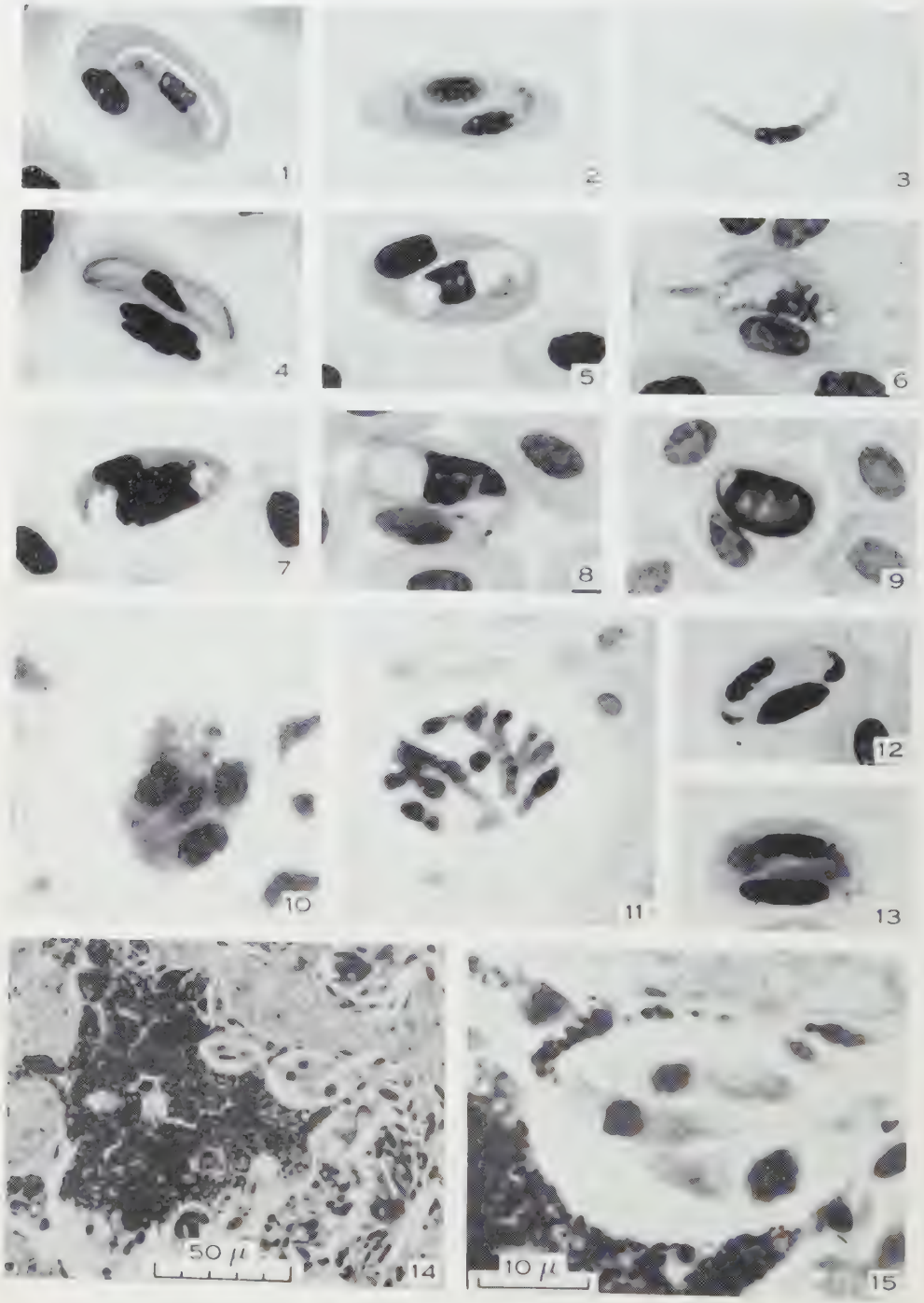

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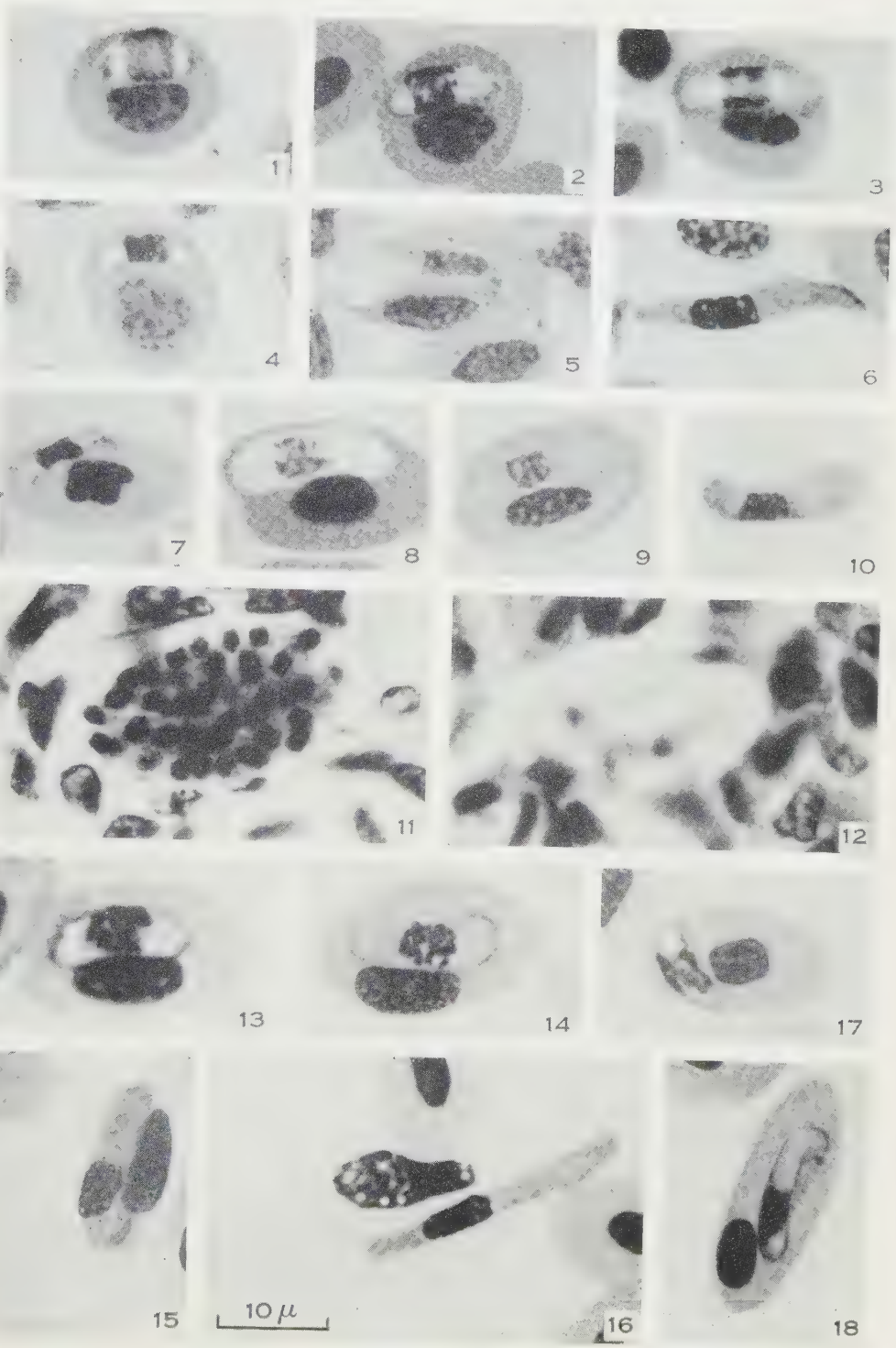

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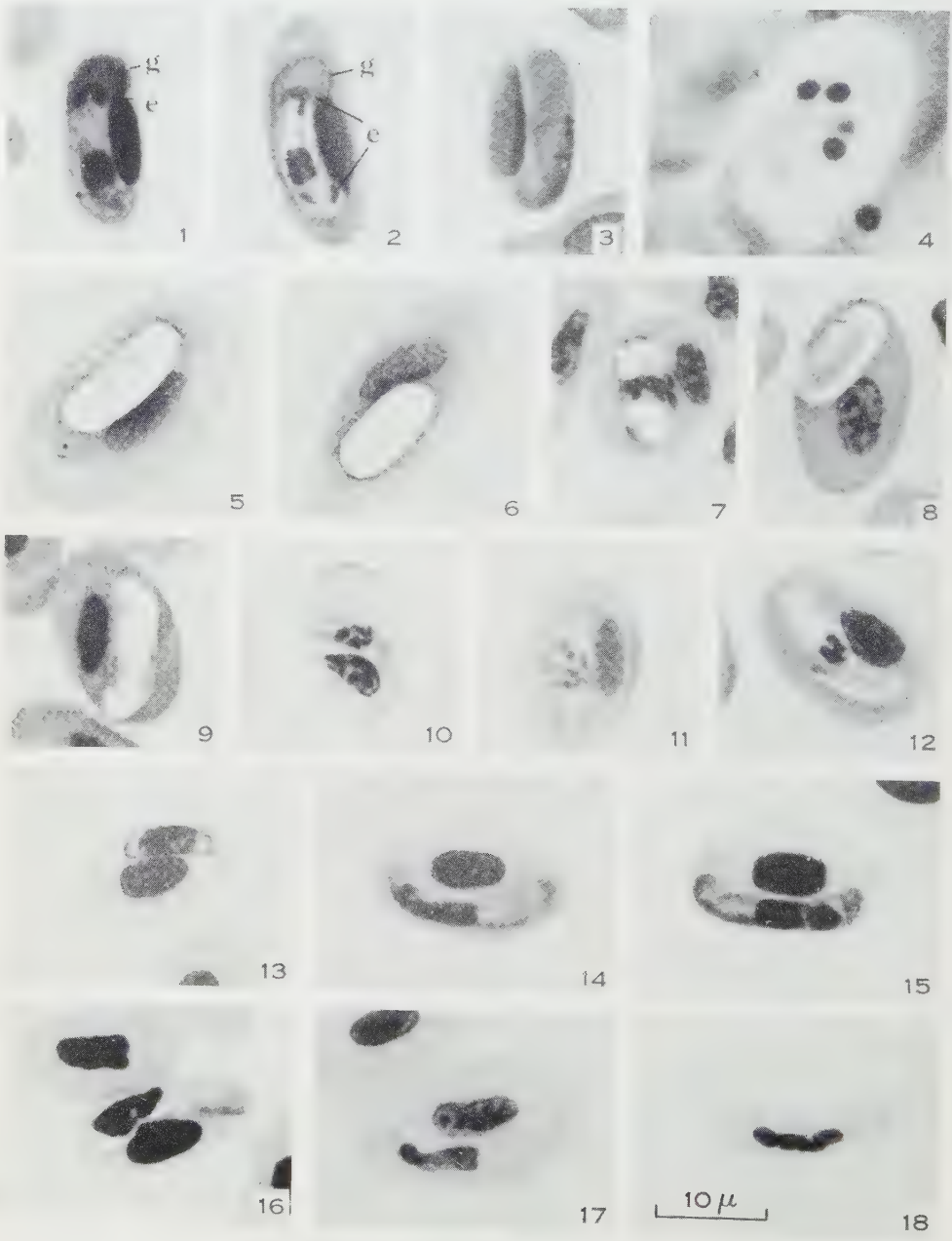

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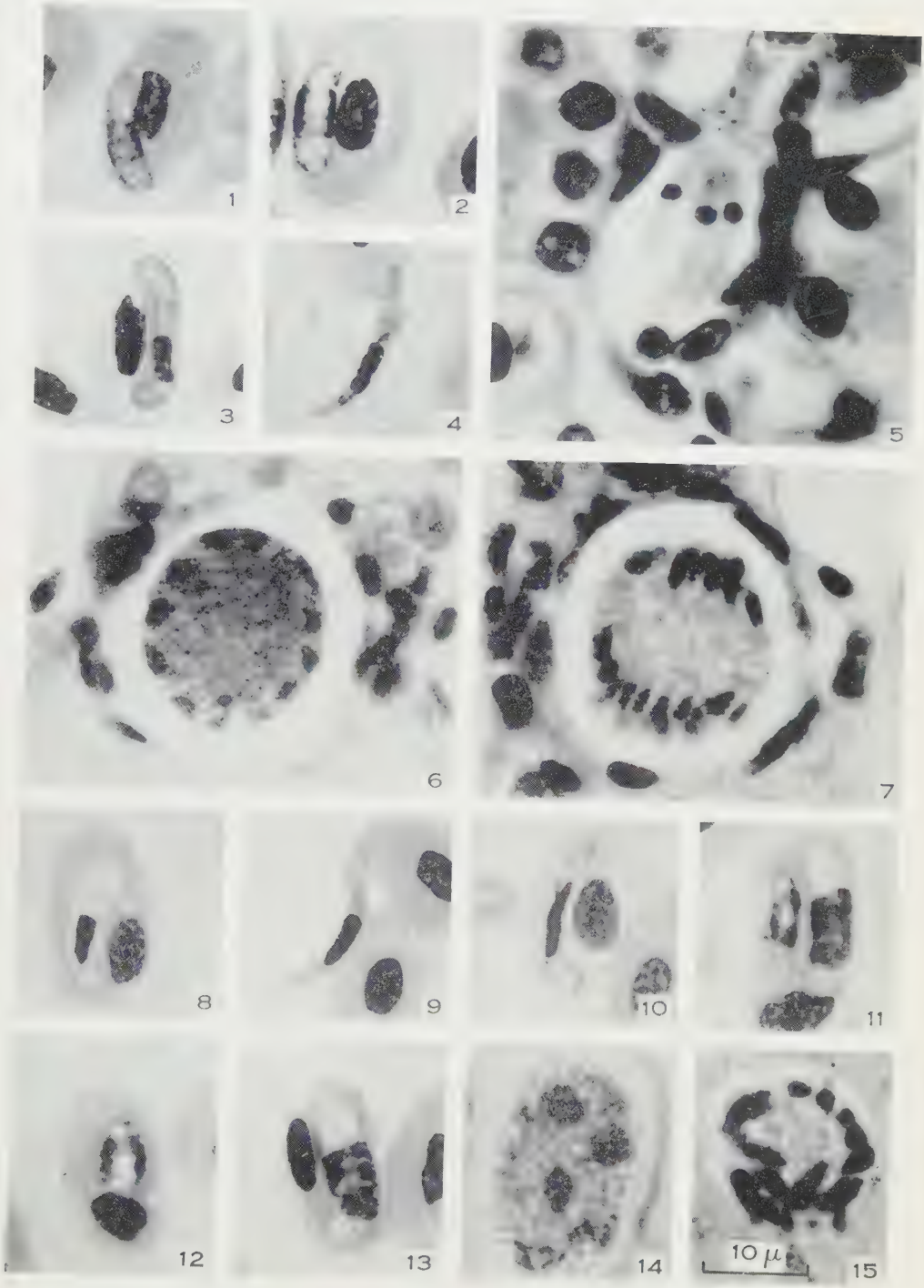

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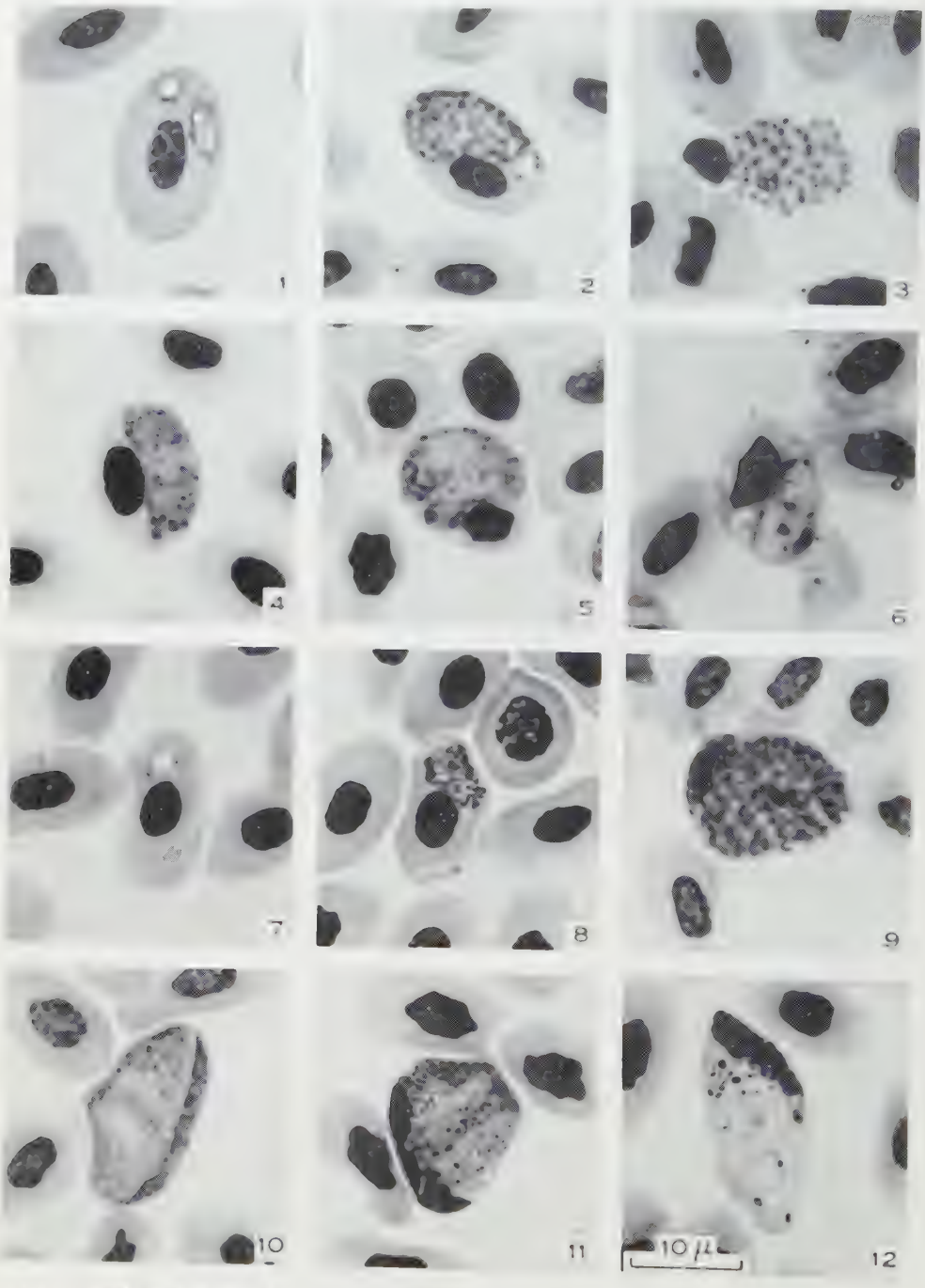

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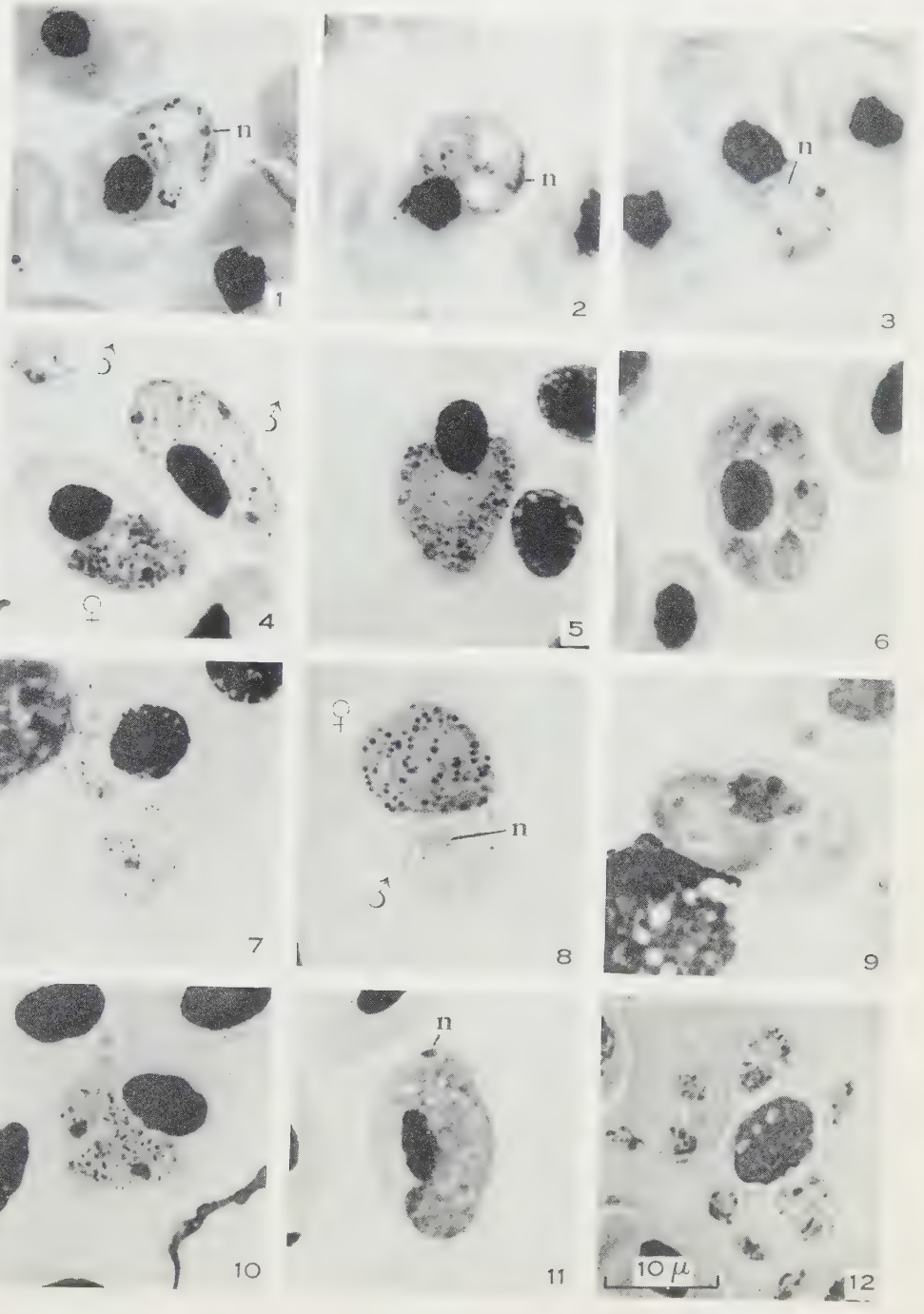

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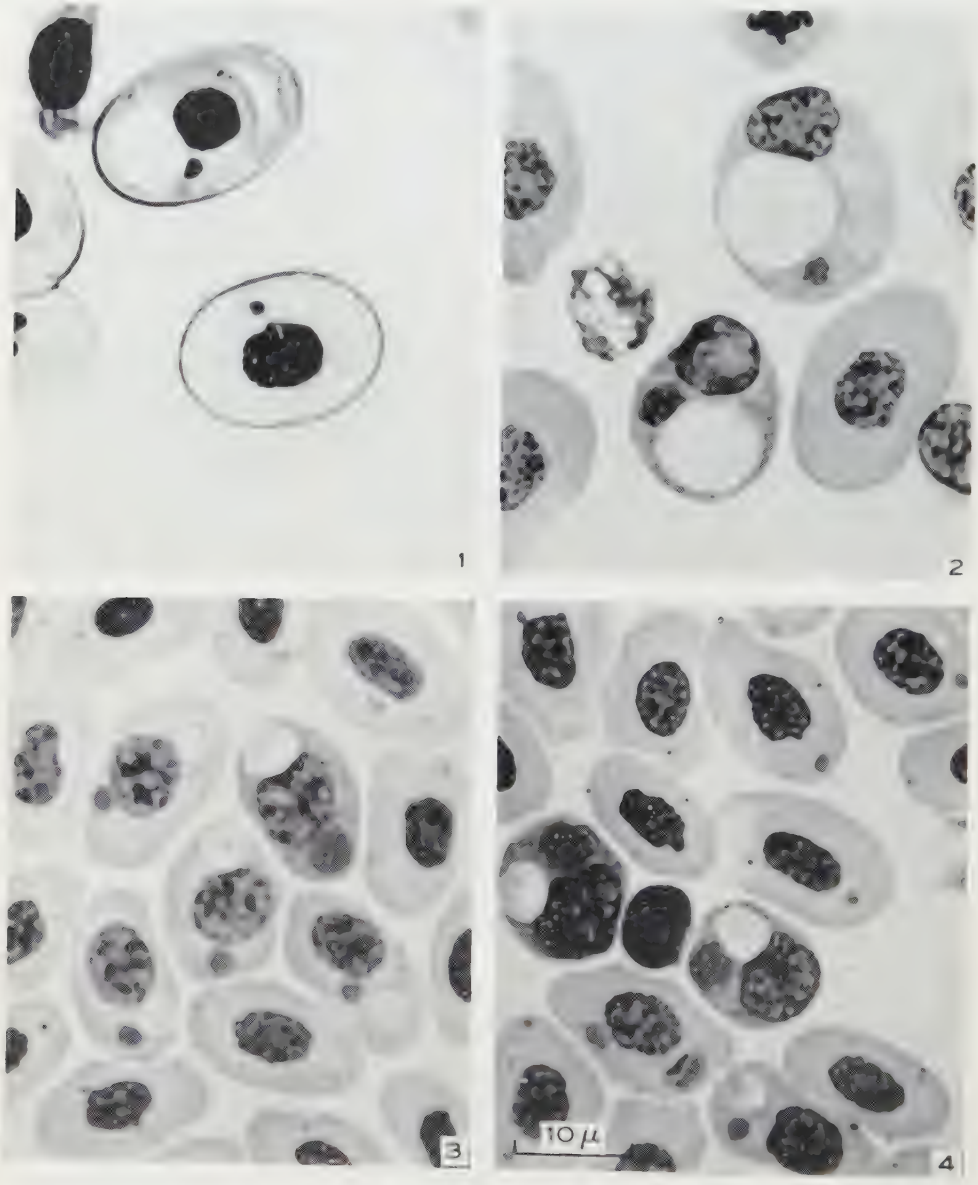

THE HAEMATOZOA OF AUSTRALIAN FROGS AND FISH

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Contents

Summary

A trypanosome and a haemogregarine have been described from frogs.

Two species of trypanosomes and 2 of haemogregarines are known from elasmobranchs; 2 trypanosomes, 1 trypanoplasm, and 4 haemogregarines from marine teleosteans; and 2 trypanosomes from freshwater teleosteans. *Haemogregarina hemiscyllii* from *Hemiscyllium ocellatum* (Elasmobranchii) and *Hg. tetraodontis* from *Tetraodon hispidus* (Teleostei) are new.

I. Introduction

Very little work has been done on the blood parasites of frogs and fish in Australia. Johnston and Cleland described two species of trypanosomes from freshwater fish in 1910 and a haemogregarine from the large green tree frog in the same year, and in 1916 Johnston named the trypanosome of the leptodactylid frogs. The present authors described several species of trypanosomes and haemogregarines and one *Trypanoplasma* from marine fish in 1925. No further publications have dealt with Australian fish, but Laird (1948–58) has studied the blood parasites of fish in various islands from New Zealand to the Gilbert and Ellice group. We have little new material, examination of the blood of many marine fish having proved unprofitable, but we have gathered together what information we have, in order to complete the review of the blood parasites of

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Australian vertebrates. The Cyclostomata are omitted, because we found no infections in four *Mordacia mordax* (Richardson), the only species examined. Wenyon (1926) has reviewed the earlier European studies of life histories on which the brief notes given in this paper are based.

II. Methods

Thin blood films were dried in the air and stained with Leishman's stain, or fixed in methanol and stained with dilute Giemsa's stain (one drop to 1 ml distilled water buffered to pH 7·4), or stained with "Soloid" eosin–azur, using the same technique as for the Leishman stain, but leaving the diluted stain on the slide for 30–60 min.

When describing trypanosomes, we have followed the nomenclature of Wenyon (1926), and have used the following abbreviations:

- L Total length of body, measured along the mid-line, and excluding the free flagellum.
- B Maximum breadth, including the undulating membrane, usually measured near the nucleus.
- PK Distance from posterior end to the kinetoplast.
- KN Distance from the kinetoplast to the posterior edge of the nucleus.
- NA Distance from the anterior edge of the nucleus to the anterior end of the body
- FF Free flagellum.

III. Host–Parasite List

Classification and Name of Host	Parasite
AMPHIBIA	
Leptodactylidae	
Limnodynastes tasmaniensis Günther, 1858	*Trypanosoma clelandi* Johnston
L. ornatus (Gray, 1842)	*Trypanosoma clelandi* Johnston
Hylidae	
Hyla nasuta (Gray, 1842)	*Trypanosoma* sp.
H. lesueurii Duméril & Bibron, 1868	*Trypanosoma* sp.
H. caerulea (Shaw, 1790), green tree frog	*Lankesterella hylae* Cleland & Johnston
ELASMOBRANCHII	
Carcharias sp., shark	*Trypanosoma carchariasi* Laveran *Haemogregarina carchariasi* Laveran
Hemiscyllium ocellatum (Bonnaterre, 1788), ocellated cat-shark	*Trypanosoma gargantua* Laird *Haemogregarina hemiscyllii*, sp. nov.
TELEOSTEI	
Aulopidae	
Aulopus purpurissatus Richardson, 1843, sergeant-baker	*Trypanosoma aulopi* Mackerras & Mackerras *Haemogregarina aulopi* Mackerras & Mackerras

Classification and Name of Host	Parasite
Plotosidae	
Tandanus tandanus Mitchell, 1838, freshwater cat-fish	*Trypanosoma bancrofti* Johnston & Cleland
Anguillidae	
Anguilla reinhardtii Steindachner, 1867, long-finned eel	*Trypanosoma anguillicola* Johnston & Cleland
A. ?mauritiana Bennett, 1831, marbled eel	*Trypanosoma anguillicola* Johnston & Cleland
Serranidae	
Ellerkeldia semicincta (Cuvier & Valenciennes, 1833), half-banded rock-perch	*Trypanosoma pulchra* Mackerras & Mackerras *Haemogregarina gilbertiae* Mackerras & Mackerras
E. annulata (Günther, 1859), banded rock-perch	*Haemogregarina gilbertiae* Mackerras & Mackerras
Pomacentridae	
Parma microlepis Günther, 1862, white-ear	*Trypanosoma pulchra* Mackerras & Mackerras *Trypanoplasma parmae* Mackerras & Mackerras *Haemogregarina parmae* Mackerras & Mackerras *Haemogregarina aulopi* Mackerras & Mackerras
Tetraodontidae	
Tetraodon hispidus Linnaeus, 1758, stars and stripes toado	*Haemogregarina tetraodontis*, sp. nov.

IV. Parasites of Frogs

Genus TRYPANOSOMA Gruby

Trypanosoma clelandi Johnston

Trypanosoma rotatorium Cleland and Johnston, 1910, p. 255; Cleland 1914, p. 413; nec Mayer, 1843.

Trypanosoma clelandi Johnston, 1916, p. 60.

Hosts.—*Limnodynastes ornatus* (Gray) (type host), *L. tasmaniensis* Günther.

Distribution.—Harrisville (type locality), Myrtletown, Eidsvold, S. Qld.; Morgan, S. Aust.

Cleland and Johnston (1910) recorded this trypanosome from slides made by Dr. T. L. Bancroft from *L. ornatus* at Harrisville and from *L. tasmaniensis* at Myrtletown, near Brisbane, provisionally allotting it to *T. rotatorium*. At the same time they pointed out that, owing to the wide geographical separation of the frogs of Australia from those of Europe and the enormous lapse of years since the hosts were associated, it was likely that the parasites were specifically distinct. In 1916, Johnston considered that the Australian species should be separated from the European one, and proposed the specific name *clelandi*.

Morphology (Plate 1, Figs. 1–3)

The trypanosomes in the frog from Harrisville were described as being similar in appearance, differing from each other mainly in breadth. The posterior ends were gradually attenuated. The prominent kinetoplast was far removed from this end, and the reddish nucleus was close in front of it. The protoplasm of the body stained a deep blue, occasionally assuming a streaked or vacuolated appearance, but no definite ribbing could be discerned. The undulating membrane was distinct, and deeply folded. The flagellum was strikingly visible, running along the edge of the membrane, usually crossing the body in its anterior portion, and ending in a well-marked free flagellum.

Dimensions (Harrisville specimens).— L, 22·6–37·7 μ; B, 3·4–8·9 μ; PK, 4·0–8·9 μ; KN, 1·8–3·5 μ; NA, 14·2–23·1 μ; FF, 7–26·7 μ.

Cleland (1914) stated that the specimens from Morgan varied from 20 μ long by 2·5 μ broad up to 52 μ long by 8·5 μ at the widest part.

Infections were found in 4 out of 17 *L. ornatus* examined at Eidsvold, but not in 12 *L. dorsalis* from the same locality, nor in 17 *L. peronii* from various localities. The organisms were fairly numerous in one frog, scanty in the other three. They agreed well with the account given by Cleland and Johnston, except that much smaller forms were also present (Fig. 1). In all forms, the posterior end was sharply pointed. The kinetoplast was a considerable distance away from the end, and usually fairly close to the small, granular nucleus, which lay in the posterior half of the body, and was usually surrounded by a clear area. The undulating membrane was well developed in the large parasites (Figs. 2 and 3), but inconspicuous in the small ones (Fig. 1). The free flagellum was usually moderately long, but in some large forms it could not be clearly distinguished.

Dimensions (in μ) of Eidsvold specimens are set out in the following tabulation:

	Large Forms	Small Forms
L	34–43	16–19
B	6–10	2·5–4
PK	9·5–11	5–7
KN	1·5–4	1–2·5
NA	20–31	4–9
FF	5–15	10–15

The small forms of *T. inopinatum* Sergent & Sergent in *R. esculenta* figured by Wenyon (1926, p. 596) are similar to the small forms observed by us in *L. ornatus*, but the large forms are distinct, the nucleus in *T. inopinatum* being a considerable distance from the kinetoplast and usually lying in the anterior half of the body.

Life History

The parasites of the European frog, *Rana esculenta*, have been shown to be transmitted by leeches. The infection has been followed through by several observers. When tadpoles were infected by leeches carrying *T. rotatorium*, small, slender trypanosomes appeared in the blood 5 or 6 days later, and grew into the

typical "tadpole trypanosomes" 25–35 μ in length. When the tadpoles became adult frogs, curious ovoid, spherical, or leaf-like forms appeared in the blood.

Trypanosoma sp.

Trypanosomes were found by Bancroft (1891) in the blood of *Hyla nasuta* and later in *H. lesueurii* (recorded by Cleland and Johnston 1910) in Queensland. They have not been seen since, although both species of frog have been examined.

Genus LANKESTERELLA Labbé

This genus was founded in 1899 for *Anguillula minima* Chaussat, 1850, a small, unpigmented parasite of the red cells of European frogs. It belongs to the suborder Eimeriidea of the Coccidia, within which it is characterized by undergoing both schizogony and sporogony in the endothelial or macrophage cells of the vertebrate host, the oocyst giving rise to numerous sporozoites, which invade red or white cells. It has been recorded so far only from frogs and birds (Lainson 1959; Mackerras and Mackerras 1960), the related parasites of reptiles being included in *Schellackia* Reichenow, 1920, which is distinguished by development in the intestinal wall and an octozoic sporocyst.

The life cycle of *L. hylae* has not been fully worked out, and it may be intermediate between the two genera, but we have included in it *Lankesterella* because it shows several points of resemblance to *L. minima*.

Lankesterella hylae Cleland & Johnston

Haemogregarina (*Lankesterella*) *hylae* Cleland and Johnston, 1910, p. 256. Type slide from *Hyla caerulea* from Sydney, in the Australian Museum, Sydney.

Host.—*Hyla caerulea* (Shaw).

Distribution.—Sydney; Brisbane, Eidsvold, S. Qld.

Cleland and Johnston found parasites in 3 out of 5 specimens of *H. caerulea* in Sydney, and Cleland (1914) found them in 1 out of 3 from Eidsvold. We have seen them in 6 out of 13 specimens at Sydney, and in 1 out of 10 taken at Brisbane, an overall incidence of about 35%.

Morphology (Plate 1, Figs. 4–9)

Cleland and Johnston described these parasites as slender, crescentic, 9–11 μ long by 1·6–3 μ wide, with pale blue cytoplasm which was usually studded with fine reddish granules. The nucleus was usually band-like and reticular, but there were peculiar variations in appearance. A bulge containing a vacuole often occurred near the middle of the parasite, and masses of chromatinic material appeared in it, being finally extruded into the cytoplasm. The parasites were in various positions in the host red cells, which were not enlarged nor distorted, although their nuclei were sometimes displaced. Double and triple infections of red cells were noted. Free forms lost their crescentic appearance.

We have seen two heavy infections and several scanty ones. The parasites agreed closely with the original description. The smallest were spindle-shaped

bodies, 6–8 μ by 2 μ (Fig. 4). The more abundant forms were rather longer, 9–11 μ by 2–3 μ, and showed the same puzzling variation in the arrangement of chromatin as noted by the original authors. In some films, particularly in those stained by Leishman's method, the cytoplasm appeared uniformly pale blue, and the nucleus appeared to be small, discrete, granular or reticular, and situated near the mid-point of the body. Frequently, however, the interior of the parasite appeared colourless, while strands or granules of reddish staining substance protruded equatorially (Figs. 5 and 6). These masses were sometimes free in the red cell cytoplasm. Although these peculiar appearances were more suggestive of artifacts than normal development, they were seen equally frequently in films made from peripheral blood, as in films kept in a moist chamber, or in blood diluted in various ways. Some very slender intracellular parasites were also seen (Fig. 7), but they were uncommon. Free parasites, which looked very similar to the slender, intracellular forms, were found in partially laked blood. Each had a small, round, nearly centrally placed nucleus, and one or more large vacuoles in the cytoplasm (Fig. 8).

Normal red cells varied in size, measuring 18–22 μ by 11–14 μ; infected cells 19–22·5 μ by 11–15 μ; they appeared unchanged, except for slight displacement of the nucleus.

Numerous free parasites were found in organ smears from the heavily infected frogs, and exoerythrocytic stages were seen in liver smears and in some from the spleen. These were in relatively small macrophages with round or elongate nuclei and clumps of pigment in the cytoplasm (Fig. 9). From 2 to 7 small parasites were observed. They were oval, crescentic, or sausage-shaped, with band-like reticular nuclei, and with 1 or 2 vacuoles. Whether they were merozoites or sporozoites could not be determined, but their similarity to the forms liberated from red cells suggests that they may have been sporozoites.

Life History

As indicated above, the whole life cycle of *Lankesterella* takes place in the vertebrate, the invertebrate host acting simply as a mechanical transmitting agent. *L. minima* is transmitted by aquatic leeches, and the parasites of birds are transmitted by mites (Lainson 1959).

V. Parasites of Elasmobranch Fish

Genus TRYPANOSOMA Gruby

Trypanosoma carchariasi Laveran

Trypanosoma carchariasi Laveran, 1908, p. 150.

Host.—*Carcharias* sp. (a shark).

Distribution.—Broughton I.*

Very scanty trypanosomes were found in blood films of a shark sent to Laveran by M. Latapie. The body was described as cylindrical, attenuated at both

* The type locality is probably Broughton I., near Port Stephens, N.S.W., but there is also a Broughton I. near Mackay, Qld.

ends, the protoplasm finely granular, and the kinetoplast near the posterior extremity. The length was given as 60–70 μ, including the free flagellum. The figure depicts a long, narrow trypanosome with sharply pointed posterior end. The small round kinetoplast lay about one-eighth of the body length from the posterior end. The nucleus was oval, lying well anterior to the mid-point of the body; the undulating membrane was narrow, with about 9 undulations; and the free flagellum measured about one-seventh of the body length. The species of shark was not determined, and the parasite has not been recorded again.

Trypanosoma gargantua Laird

Trypanosoma gargantua Laird, 1951, p. 293. Type slide from *Raja nasuta* from Cook Strait, N.Z., in the Dominion Museum, Wellington.

Hosts.—*Raja nasuta* Müller & Henle (a skate), *Hemiscyllium ocellatum* (Bonnaterre).

Distribution.—Cook Strait, N.Z.; Heron I., Qld.

Morphology (Plate 2, Fig. 1)

We have seen a single large trypanosome, which seems to be referable to this species, in a blood film from *H. ocellatum* from Heron I. It is evidently an uncommon parasite in these sharks, as examination of films from 55 other specimens from Heron I. and Low I. failed to reveal another infection.

The parasite was lying in the C position, with the anterior extremity close to the body, so that the presence or absence of a free flagellum could not be determined. It fits Laird's description of the parasites of the New Zealand skate fairly closely, the only difference being in the position of the kinetoplast, which was further away from the posterior end than in Laird's specimens. Very numerous, fine myonemes were present, clearly seen where they crossed the nucleus (Fig. 1). Dimensions (μ) are given in the following tabulation:

	T. gargantua from *R. nasuta*	Form from *H. ocellatum*
L	66·7–131·1 (av. 114·7)	105
B (without membrane)	6·7–17·1 (av. 14·3)	15
Undulating membrane	1·0–3·4 (av. 2·9)	2
PK	13–25% of L	41 (39% of L)
KN		21
NA	33.7–42% of L	42 (40% of L)
Nucleus	3·9–9·3 by 3·3 6·7	10 by 7
Karyosome (diameter)	2·9–5·2	4
FF	0	?

Life History

The trypanosomes of European rays are carried by marine leeches, *Pontobdella muricata* being the vector of *T. rajae* Laveran & Mesnil and *T. giganteum* Neumann. Laird (1951) noted that a pontobdellid leech has been recorded on *R. nasuta*, but he was unable to obtain specimens from infected rays. We have not seen leeches on *H. ocellatum*.

Genus HAEMOGREGARINA, s.l.

The classification of the haemogregarines has been discussed in the preceding paper (Mackerras 1961). Wenyon (1926) was inclined to include the species from fish in the restricted genus *Haemogregarina* Danilewsky, 1885. This may be true of those from elasmobranchs, but so little is known of their development that we prefer to treat them as broadly as was done with the parasites of reptiles.

Haemogregarina carchariasi Laveran

Haemogregarina carchariasi Laveran, 1908, p. 150.

Host.—Carcharias sp.

Distribution.—Broughton I.

Laveran described this species from the same films that contained *Trypanosoma carchariasi*. The parasites were cylindrical with rounded ends, or oval with one extremity more rounded than the other. They measured 20–27 μ by 7–10 μ, the cytoplasm contained basophilic granules, and the rounded nucleus lay in the middle of the body. Dividing forms and free vermicules were not seen. The infected cells were elongated, up to 34 μ long, whereas normal red cells were 26 μ by 14–15 μ. The nucleus of the infected cell was not hypertrophied.

Haemogregarina hemiscyllii, sp. nov.

Host.—Hemiscyllium ocellatum (Bonnaterre).

Distribution.—Heron I., ?Low I., Qld.

Type.—Slide from *H. ocellatum* from Heron I., in the Queensland Museum, Brisbane.

We have seen 2 moderately abundant and 3 very scanty infections in 48 *H. ocellatum* from Heron I., and one doubtful infection in 8 from Low I.

Morphology (Plate 3, Figs. 1–4)

The smallest parasites seen were slightly curved bodies with rounded ends, measuring 13–14 μ by 2 μ, and occupying small red cells. The nucleus was heavily stained, and filled about one-half of the body. Most of the parasites were much larger, 15–19 μ by 4–5 μ, with a large heavily stained nucleus occupying one-half to two-thirds of the body (Fig. 1). The cytoplasm was pale blue or colourless, and sometimes contained a few fine, reddish granules. These were probably trophozoites. Some larger parasites, 16–19 μ by 5–8 μ, contained much less chromatin, which was sometimes arranged mitotically (Figs. 2 and 3), but no actually dividing forms were found. Sometimes the nucleus did not extend across the full width of the body (Fig. 4), and a few parasites were almost spherical. They may have been doubled up vermicles, but a capsule was not distinguished around any of them, and no free vermicules were seen. A single parasite found in a specimen from Low I. was a slender curved body, with pointed ends, and a small central nucleus. It may be a distinct species. Red cells varied greatly in maturity and in size, ranging from 17–24 μ by 10–15 μ; infected cells ranged from 17–24 μ by 14–18 μ.

This parasite is distinct from *Hg. carchariasi*, which is much longer, and has a relatively small, centrally placed nucleus.

VI. Parasites of Teleostean Fish

Genus TRYPANOSOMA Gruby

Trypanosoma pulchra Mackerras & Mackerras

Trypanosoma pulchra Mackerras and Mackerras, 1925, p. 361. Type slide from *Ellerkeldia semicincta* from Sydney Harbour, in the Australian Museum, Sydney.

Hosts.—Ellerkeldia semicincta (Cuvier & Valenciennes), *Parma microlepis* Günther.

Distribution.—Sydney Harbour and Broken Bay, N.S.W.

This species was found in 3 out of 38 *E. semicincta* and in 1 (possibly 2) out of 9 *P. microlepis*.

Morphology (Plate 2, Fig. 3)

This was a long slender trypanosome, with a well-developed undulating membrane, and a short free flagellum. The cytoplasm stained deeply, showing irregular lighter and darker areas, and a number of small, scattered vacuoles. The posterior end was beak-shaped, with the kinetoplast near it, and the nucleus, which stained uniformly pink, was near the centre of the body (Fig. 3). The trypanosomes in one *P. microlepis* could not be separated morphologically from those in *E. semicincta*, and, although the hosts are not closely related, they are found in the same situations, and may have the same ectoparasites.

Dimensions (average).—L, 48·3 μ (range 40·8–57·1 μ); B, 3·5 μ; PK, 1·7 μ; KN, 20·8 μ; FF, 7·3 μ.

Two small trypanosomes were found among the ordinary forms in one *E. semicincta*. They measured 26.5 and 27.2 μ, and possessed dark granular nuclei. In other respects they looked like small replicas of *T. pulchra*. A single large trypanosome was found in the second *P. microlepis*. It appeared broader and less graceful than *T. pulchra*. Its nucleus lay slightly posterior to the mid-point, and the cytoplasm contained fine granules (Fig. 5). Its dimensions were: L, 51 μ; B, 5 μ; PK, 1·5 μ; KN, 21 μ; NA, 25 μ; FF, 6·5 μ; nucleus 3·5 by 2 μ.

Trypanosoma aulopi Mackerras & Mackerras

Trypanosoma aulopi Mackerras and Mackerras, 1925, p. 362. Type slide from *Aulopus purpurissatus* from Sydney Harbour, in the Australian Museum, Sydney.

Host.—Aulopus purpurissatus Richardson.

Distribution.—Sydney Harbour.

It was found in 1 out of 8 specimens examined.

Morphology (Plate 2, Fig. 4)

A long slender species (Fig. 4), very similar to the large forms of *T. pulchra*, but distinguished by marked pleomorphism. Total length ranged from 29 to 57 μ.

It was separated from *T. pulchra* more on account of the wide taxonomic separation of the hosts, than on any constant morphological feature.

Trypanosoma bancrofti Johnston & Cleland

Trypanosoma bancrofti Johnston and Cleland, 1910, p. 407. Type slide from *Tandanus tandanus* from Kilcoy, in the Australian Museum, Sydney.

Host.—*Tandanus tandanus* Mitchell.

Distribution.—Kilcoy, Eidsvold, Cairns, Qld.

Johnston and Cleland described this species from films made by Dr. T. L. Bancroft in 1905 and 1910. It was present in 1 out of 10 freshwater cat-fish (called "jewfish" locally) obtained near Kilcoy.

Morphology (Plate 2, Fig. 2)

It was described as a pleomorphic species, most abundant forms being comparatively short and very narrow, but some much longer, broader forms also being present. The kinetoplast was close to the posterior end, and the nucleus near the mid-point. The undulating membrane was narrow, and the free flagellum rather variable. Three forms of the Kilcoy specimens were present in the type slide: (1) moderately numerous, narrow forms; (2) one very broad form containing deep blue granules; (3) one intermediate form containing scattered basophilic granules. Their dimensions (μ) are given below:

	Form (1)	Form (2)	Form (3)
L + FF	27–31	50	34
B	2–3	7	3·5
PK	1	1·8	
KN	8–12·5	12·5	
NA	9	11	
FF	4–11	21·5	

We have studied a very abundant infection in a jewfish taken from Freshwater Creek, near Cairns. From 1 to 5 parasites were present in nearly every oil-immersion field. The organisms were very uniform in size, and no dividing forms were seen. The kinetoplast was situated at the posterior extremity; it was rod- or V-shaped, and often projected posteriorly (Fig. 2). The nucleus was oval, about 3 by 1·5 μ, and situated at about the mid-point of the body. The undulating membrane was narrow, and the free flagellum very fine and of moderate length.

Dimensions (Cairns specimens).—L, 21·5–25 μ; L + FF, 30–36·5 μ; B, 2–2·5 μ; PK, 0; KN, 10·5–11·5 μ; NA, 7·5–10 μ; FF, 9–11·5 μ.

Trypanosoma anguillicola Johnston & Cleland

Trypanosoma anguillicola Johnston and Cleland, 1910, p. 406. Type slide from *Anguilla reinhardtii* from southern Queensland, in the Australian Museum, Sydney.

Hosts.—*Anguilla reinhardtii* Steindachner, *A. ?mauritiana* Bennett.*

* The specific identity of this eel is doubtful. Mr. N. Haysom (personal communication) says that *A. mauritiana* has not so far been definitely identified from Australia.

Distribution.—Burnett R. near Eidsvold and unnamed localities, S. Qld.; Prospect Reservoir, N.S.W.

Johnston and Cleland described this species from films made in various localities in southern Queensland by Dr. T. L. Bancroft in January and May, 1910, from *A. reinhardtii*, and from *A. ?mauritiana* from the Burnett R., near Eidsvold. They also found a single trypanosome in 1 out of 4 *A. reinhardtii* from the Prospect Reservoir.

Morphology (Plate 2, Fig. 6)

This species was described as a long narrow trypanosome, with the body gradually attenuated towards each end; the cytoplasm was deeply stained by Giemsa, but apparently free from granules; the kinetoplast was near the posterior end; and the nucleus was near the centre, or anterior to it. The undulating membrane was well developed, with numerous undulations, and the free flagellum was very short.

Dimensions (type series).—L + FF, 35·5–40 μ; B, 2·5 μ; PK, 1·5 μ; KN, 16–28 μ; FF, 3·7–7 μ.

We have seen a scanty infection in the blood of an *A. reinhardtii* from the Burnett R. Some of the parasites were larger than those described by Johnston and Cleland, the flagellum was longer, and the nucleus was situated in the posterior half of the body (Fig. 6). The dimensions of the specimen photographed were: L, 44 μ; B, 3 μ; PK, 2 μ; KN, 17 μ; NA, 21 μ; FF, 18 μ; nucleus, 2·5 by 1·5 μ.

This is evidently close to *T. granulosum* Laveran & Mesnil, 1902, in European eels. It is distinguished by its smaller size (*T. granulosum* reaches a total length of 80 μ, of which the body measures 55 by 2·5–3 μ, and the flagellum 25 μ), and by the lack of granules in the cytoplasm.

Life History

T. granulosum has been shown to develop in a leech, *Hemiclepsis marginata*. Multiplication took place in the stomach, and the flagellates migrated forward and congregated in the proboscis sheath. Infection apparently occurred during biting.

Genus TRYPANOPLASMA Laveran & Mesnil

This genus is not closely related to *Trypanosoma*. The kinetoplast is anterior, and there is a single anterior flagellum and a trailing one which is attached to the body by an undulating membrane. Some authorities have regarded it as a synonym of *Cryptobia* Leidy, 1846, but it seems reasonable to use *Trypanoplasma* for the blood-dwelling parasites of fish, leaving *Cryptobia* for intestinal parasites.

TRYPANOPLASMA PARMAE Mackerras & Mackerras

Trypanoplasma parmae Mackerras and Mackerras, 1925, p. 362. Type slide from *Parma microlepis* from Sydney Harbour, in the Australian Museum, Sydney.

Host.—Parma microlepis Günther.

Distribution.—Sydney Harbour.

Morphology (Plate 2, Figs. 7 and 8)

This species was present in small numbers in 3 out of 9 fish examined.

It was monomorphic, rather broad, normal specimens measuring from 12·5 μ long by 3·8 μ wide to 14·7 μ by 5·0 μ (Fig. 7). The cytoplasm stained a faint blue, and was finely and irregularly granular. It was pale and indistinctly vacuolated anteriorly, denser posteriorly, where it contained numerous, round or irregular, chromatoid bodies, 0·25–1·25 μ or occasionally more in diameter, which sometimes extended round the anterior margin of the nucleus. A narrow zone of densely packed, fine, reddish granules extended along the dorsal edge of the body, and marked the origin of the undulating membrane. The nucleus was behind the middle of the body, reniform or occasionally oval in shape, 3·0–3·7 μ by 1·5–2·3 μ, and fairly densely packed with large, irregular, chromatinic masses. The kinetoplast was a large, oblong, or oval mass, 1·7–2·0 μ by 1·0–1·3 μ, situated close to the ventral surface, about 2 μ from the anterior end; it stained an intense reddish black. The blepharoplast was a minute darkly stained granule close to the anterior extremity of the body; it usually appeared to be single, but was occasionally double. No definite rhizoplast was seen. The anterior free flagellum was 18–25 μ long. The posterior flagellum was attached to the body by an undulating membrane little more than 0·25 μ wide; its free terminal part measured 18·0–26·8 μ.

Many specimens were abnormally rounded, measuring 9·5–11 μ by 7–8·5 μ (Fig. 8). Some were highly vacuolated, giving the impression that they were about to disintegrate.

We have not had the opportunity to examine more material; but, in retrospect, we feel that we may not have exercised enough care to prevent contamination of the blood with fluid from the oesophagus, so that *Tp. parmae* may possibly be a parasite of the alimentary tract and not of the blood. The rather patchy distribution of parasites in some of the films and the tendency to distortion would be in keeping with a contaminative origin.

Life History

The trypanoplasms of several freshwater fishes have been shown to be transmitted by leeches.

Genus HAEMOGREGARINA, s.l.

The haemogregarines of marine teleosts are mostly small organisms. Multiple infections of cells are common, and many of the species appear to multiply by successive binary fission, a characteristic which would set them apart from the haemogregarines of higher vertebrates. Their classification has been discussed by Laird (1952, 1953), but we have been unable to correlate our rather meagre findings fully with his, and it seems likely that the group may ultimately be divided into several distinct genera. Nothing is yet known of their development in the invertebrate, or method of transmission.

Haemogregarina gilbertiae Mackerras & Mackerras

Haemogregarina gilbertiae Mackerras and Mackerras, 1925, p. 365. Type slide from *Ellerkeldia semicincta* from Sydney Harbour, in the Australian Museum, Sydney.

Hosts.—Ellerkeldia semicincta (Cuvier & Valenciennes), *E. annulata* (Günther).

Distribution.—Sydney Harbour, Broken Bay, N.S.W.

The parasites were present in 4 out of 38 *E. semicincta* and 1 out of 3 *E. annulata*. These fish were originally placed in the genus *Gilbertia*, hence the specific name of the parasite.

Morphology (Plate 3, Figs. 5 and 6)

The organisms were broadly oval, fairly uniform in size, 3·5–5·6 μ by 2·5 μ, most being about 4·2 by 2·5 μ. The chromatin usually formed a loose skein at one end, sometimes widely spread in the form of granules. A free parasite measured 10·4 by 1·9 μ, with pale greyish blue cytoplasm, and the chromatin collected into two masses towards one end. Most infected cells contained two parasites (Fig. 6), but single infections (Fig. 5) were also found, and once three parasites were seen in a red cell. The infected cells were slightly enlarged. Normal cells varied from almost round disks about 8 μ in diameter to oval forms measuring 8–10 μ by 5–7 μ; infected cells measured 9–10·5 μ by 6–9 μ. The cytoplasm sometimes stained more darkly than normal, and the nuclei were sometimes slightly displaced.

Haemogregarina parmae Mackerras & Mackerras

Haemogregarina parmae Mackerras and Mackerras, 1925, p. 365. Type slide from *Parma microlepis* from Sydney Harbour, in the Australian Museum, Sydney.

Host.—Parma microlepis Günther.

Distribution.—Sydney Harbour.

It was found in 7 out of 9 *P. microlepis* examined.

Morphology (Plate 3, Figs. 7–9)

Trophozoites and gametocytes were recognized. The former measured 6·2 9·6 μ by 1·2–1·7 μ, the body being delicate in outline, usually somewhat curved, and tapering more to one end than the other. The nucleus lay near the middle, with strands and granules extending into the more attenuated end. From 1 to 4 parasites were commonly found (Fig. 7), and rarely 6 or 7 in a red cell; these may have been the product of successive divisions.

The gametocytes were more definite in outline. Both ends were rounded, or, in forms about to leave the cell, the posterior end was pointed and the anterior broadly rounded. These intracellular forms were about 7·1 by 1·9 μ (Fig. 8). The free vermicules were about 11·5 by 2·3 μ, the largest being 13·4 by 2·9 μ. The cytoplasm was pinkish, blotchy, and the anterior end was darkly stained (Fig. 9). The chromatin sometimes extended throughout the posterior half of the body, or was collected into a mass of blocks and granules near the middle.

Infected cells were distinctly enlarged. Normal cells measured 8·5–11 μ by 5·5–8 μ; infected cells 11–12 μ by 7·5–9 μ; their nuclei were not usually displaced.

Haemogregarina tetraodontis, sp. nov.

Host.—Tetraodon hispidus (Linnaeus).

Distribution. Mornington I., Gulf of Carpentaria; Low I., near Port Douglas, Qld.

Type.—Slide from *T. hispidus* from Mornington I. in the Queensland Museum, Brisbane.

We have seen a rather scanty infection in a single specimen of *T. hispidus* examined at each of the localities mentioned above.

Morphology (Plate 3, Figs. 10–14)

The smallest parasites seen in the type slide were short ovoid forms, about 4 by 2 μ, lying singly in the infected cells, which were not enlarged (Fig. 10). The largest single parasite was 8 by 2 μ. Division appeared to be by longitudinal binary fission, producing a pair of parasites at first closely approximated. Sometimes both members divided again (Figs. 11 and 13), sometimes only one, giving rise to three elongate forms (Fig. 12). Each of these sometimes divided again, producing six small parasites about 4 by 1 μ (Fig. 14). The maximum number seen in a red cell was six. The parasite nucleus was relatively large, occupying about half the body. No vermicules were detected. The infected cells, except those containing single parasites, were definitely enlarged. Normal cells measured 7·5–11 μ by 6–8 μ; infected cells 9–12·5 μ by 7–10 μ. The parasites seen in the fish from Low I. were slender, elongate forms, usually lying in a compact bundle beside the host cell nucleus, and difficult to resolve into individuals.

This species differs from *Hg. parmae* in being broader, and in causing more enlargement of the red cell and displacement of its nucleus.

Haemogregarina aulopi Mackerras & Mackerras

Haemogregarina aulopi Mackerras and Mackerras, 1925, p. 363. Type slide from *Aulopus purpurissatus* from Sydney Harbour, in the Australian Museum, Sydney.

Hosts.—Aulopus purpurissatus Richardson, *Parma microlepis* Günther.

Distribution.—Sydney Harbour.

The parasites were present in 1 out of 8 *A. purpurissatus,* and in 2 out of 9 *P. microlepis.*

Morphology (Plate 3, Figs. 15 and 16)

Small parasites of various shapes were usually applied closely to the red cell nucleus (Figs. 15 and 16). Slender forms 4·7 by 1·5 μ occurred in pairs, rounded forms 2·3–3·3 μ by 1·8–2·5 μ from 1 to 4 in a cell, and large, oval forms 4·5 by 2·9 μ occurred singly. The chromatin was usually collected into blocks at the periphery of the parasite.

These peculiar parasites are evidently similar to those seen by Henry (1910, 1913) in *Cottus bubalis* and *C. scorpius* from the Irish Channel, and in *Solea vulgaris* from Plymouth. In his first publication, he proposed the name *Haemohormidium cotti* for the parasites in *Cottus* spp. in a list, but he did not describe or illustrate them. Later (1913), he published a full description with figures, but did not mention his previous names, and concluded that the organisms were developmental stages of *Haemogregarina cotti* Brumpt & Lebailly, 1904. *Haemohormidium* was therefore a nomen nudum, but Wenyon (1926) validated it by giving a recognizable account of it under "Intracellular Structures of Doubtful Nature". It seems to us to be a true parasite, but we doubt that it is a haemogregarine.

The objects recorded by Johnston and Cleland (1909) in the red cells of a leather-jacket, *Monocanthus* sp., taken off Broughton I., N.S.W., in 1907, may be related. They were, however, considerably smaller, 0·8–1·5 μ in diameter, and the authors considered that they might be centrosomes.

VII. Acknowledgments

We are indebted to Mr. G. Mack, Queensland Museum, and to Mr. N. Haysom, Fisheries Branch, Department of Harbours and Marine, Brisbane, for assistance with the names of animals, and to Mr. I. Cook of this Institute for numerous blood films from sharks.

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EXPLANATION OF PLATES 1–3

PLATE 1

Blood parasites of frogs

Figs. 1–3.—*Trypanosoma clelandi* in *Limnodynastes ornatus*: 1, small form; 2 and 3, large forms.

Figs. 4–9.—*Lankesterella hylae* in *Hyla caerulea*: 4 and 5, small and large spindle-shaped forms; 6, large spindle-shaped form showing extrusion of chromatin; 7, slender intracellular form; 8, two free forms in partially laked blood; 9, exoerythrocytic forms in macrophage in liver smear (Schaudinn–Delafield's haematoxylin).

PLATE 2

Haemoflagellates of fish

Fig. 1.—*Trypanosoma gargantua* in *Hemiscyllium ocellatum*.

Fig. 2.—*T. bancrofti* in *Tandanus tandanus*.

Fig. 3.—*T. pulchra* in *Ellerkeldia semicincta*.

Fig. 4.—*T. aulopi* in *Aulopus purpurissatus*.

Fig. 5.—*Trypanosoma* sp. in *Parma microlepis*.

Fig. 6.—*T. anguillicola* in *Anguilla reinhardtii*.

Figs. 7 and 8.—*Trypanoplasma parmae* in *Parma microlepis*.

PLATE 3

Haemogregarines of fish

Figs. 1–4.—*Haemogregarina hemiscyllii* in *Hemiscyllium ocellatum*.

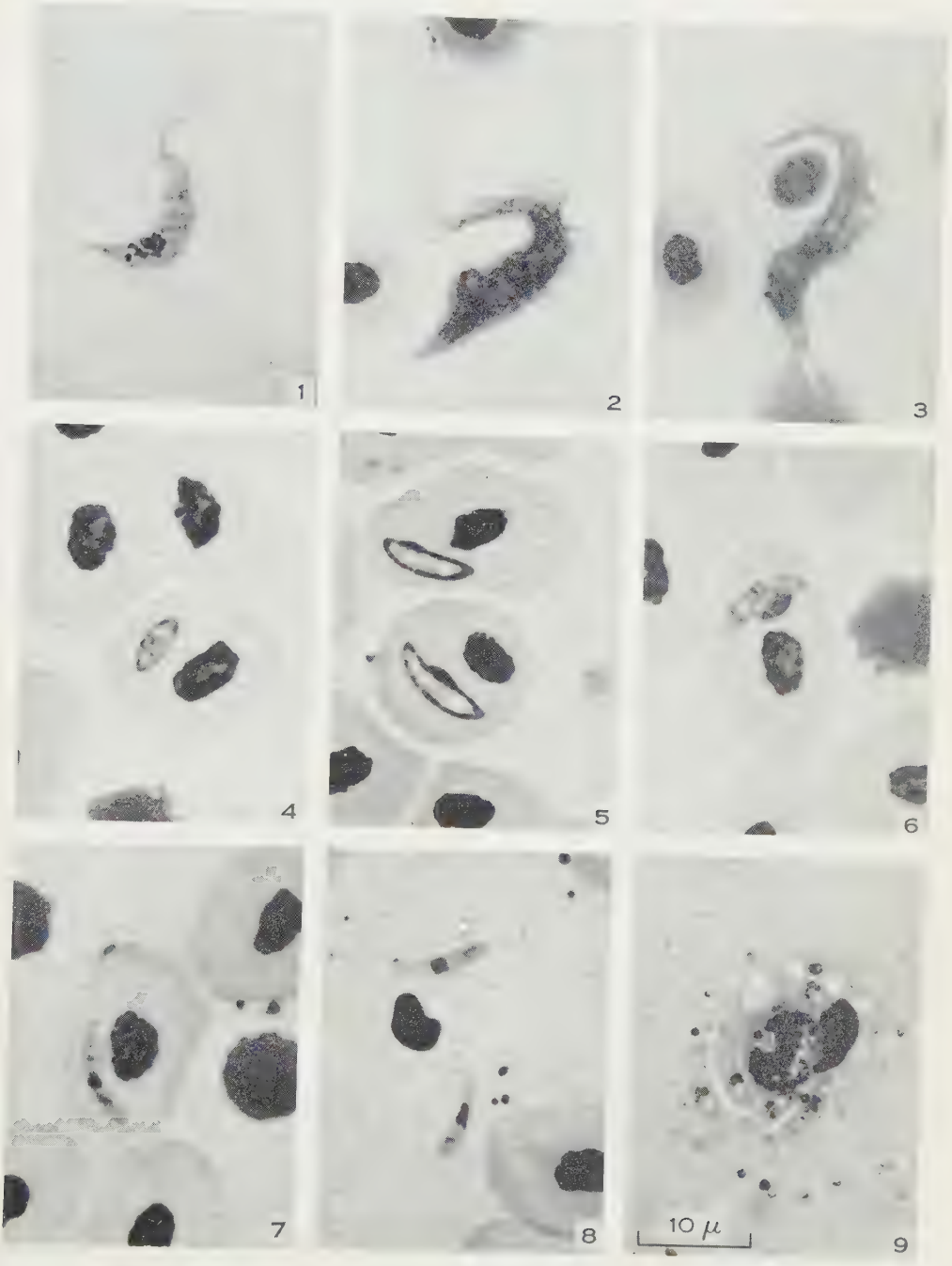
Figs. 5 and 6.—*Hg. gilbertiae* in *Ellerkeldia semicincta*.

Figs. 7–9.—*Hg. parmae* in *Parma microlepis*: 7, trophozoites; 8, intracellular vermicule; 9, free vermicule.

Figs. 10–14.—*Hg. tetraodontis* in *Tetraodon hispidus*.

Figs. 15 and 16.—*Hg. aulopi* in *Aulopus purpurissatus*.

THE HAEMATOZOA OF AUSTRALIAN FROGS AND FISH



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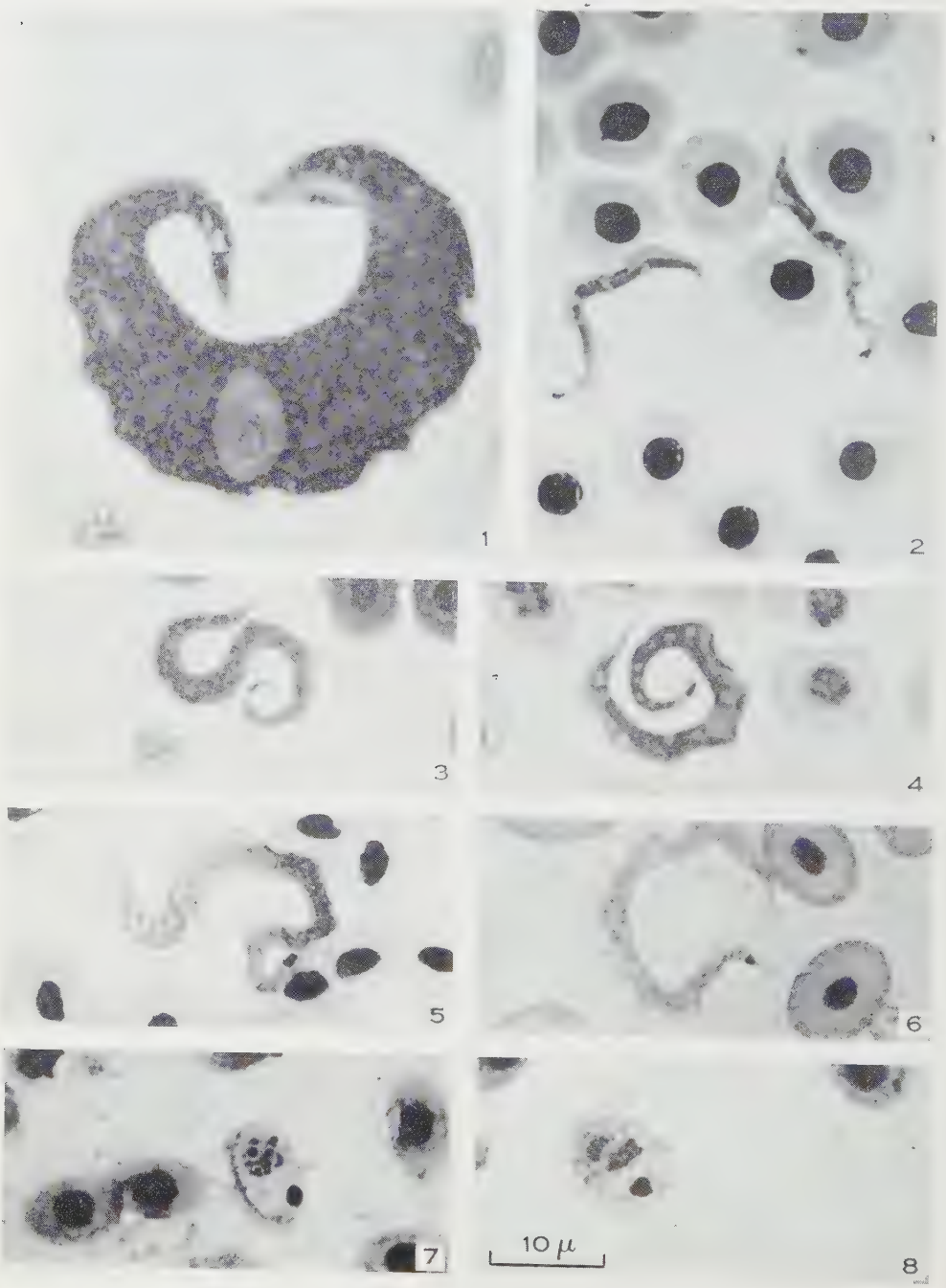
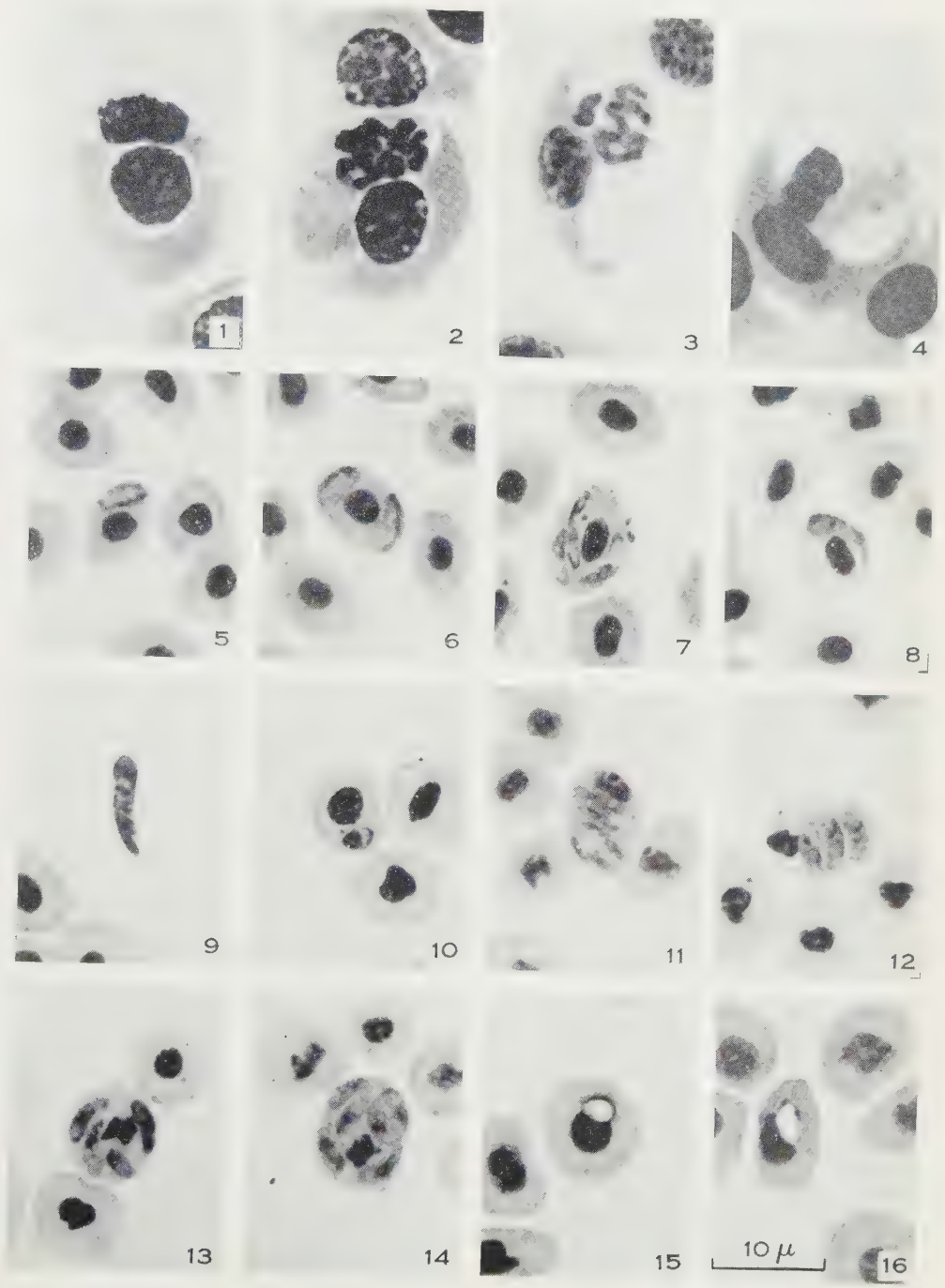


THE HAEMATOZOA OF AUSTRALIAN FROGS AND FISH



Index of Generic and Specific Names

A REVIEW OF THE AUSTRALIAN SYMPHYLA (MYRIAPODA)

By U. Scheller*

[*Manuscript received March* 8, 1960]

Contents

Summary

The present study consists mainly of taxonomic diagnoses of new species of Symphyla from a collection sent to the present author from the collector, Dr. G. F. Bornemissza, Division of Entomology, C.S.I.R.O., Canberra.

The collection consists of 285 specimens from the south-western, northern, and north-eastern parts of the Australian continent.

The material studied here contains representatives of 13 species. All but one are new. Two of them are in hand only as inadequate material and have not been described. The additional two species known from Australia have been listed in their appropriate systematic position among the others.

Family Scolopendrellidae is reported from Australia for the first time. More than four-fifths of the collection belongs to this family which seems to be common in suitable biotopes. Two genera have been found: *Scolopendrellopsis* with one species and *Symphylella* with six species, of which four are described below. The remaining two species are unnamed.

Family Scutigerellidae consists for the most part of five *Hanseniella* species of which four are new. Besides, there is a new member of the rare genus *Scolopendrelloides*. The generic diagnosis of *Scolopendrelloides* given by Bagnall has been modified.

To the extent that we have satisfactory knowledge of the distributional areas of the reported genera it is evident that the Australian species belong to very old and widespread genera but their species are all endemic and seem to have more restricted areas.

Introduction and Materials

The present work is the result mainly of a taxonomic study based on an important collection of Symphyla from the Australian continent. The treated

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material which is far richer in specimens and species than all earlier collections from this part of the world has been brought together and put at my disposal by Dr. G. F. Bornemissza, Division of Entomology, C.S.I.R.O., Canberra. This collection is very interesting and valuable from many points of view. Bornemissza has collected his material, altogether 285 specimens, from very different geographical regions. There are samples from the south-western sclerophyll and scrub woodland (Mount Toolbrunup and Gnangara), where the climate is of a Mediterranean type, and from the temperate southernmost part of Victoria (Gunyah). But there are also samples from the northern part of the continent both from the sclerophyll woodland with tropical monsoon climate in the northernmost part of Western Australia (Kimberley), and from the tropical rain-forest districts in north-eastern Queensland (Baron Falls and Hinchinbrook I.). From the southern temperate regions Symphyla have been collected and described earlier (Attems 1911; Tillyard 1930; Tiegs 1939; Scheller 1954) but this is the first time we meet the tropical forms.

Some of Bornemissza's samples were collected *in situ* (Gunyah, Baron Falls, and Hinchinbrook I.) but the rest of them from soil samples extracted by means of modified Berlese funnels. With the first method the larger and swifter forms are easily obtainable and this method was the only one used on Australian Symphyla before Bornemissza. This has meant that all earlier finds have contained only species belonging to the family Scutigerellidae. However, the studied collection mainly consists of members of the family Scolopendrellidae as more than four-fifths of the total number of the specimens can be placed in the genera *Scolopendrellopsis* and *Symphylella*.

The type material has been deposited in the collections of the Division of Entomology Museum, C.S.I.R.O., Canberra.

Taxonomic Results

All the species found are listed, and detailed taxonomic descriptions of the new forms are given. And as our knowledge of the Australian Symphyla is very slight, the following specifications are given: geographic locality, habitat, and date and number of specimens collected (adults and the different juvenile stages separately). In addition to the 13 species found by Bornemissza two more species are known to occur in Australia. They too are included in the list in their appropriate systematic position.

Key to Australian Families of Symphyla

Paired sense calicles with smooth margin of pit; terga either with pointed posterior projections, or reduced in size; styli at base of legs small or reduced to slight protuberances; first pair of legs less than half as long as following pair; length usually less than 4 mm ..... Scolopendrellidae Bagnall

Paired sense calicles with many setae around margin of pit; terga large with rounded posterior margins; styli large; first pair of legs shorter but more than half as long as following pair; length usually more than 4 mm ..... Scutigerellidae Bagnall

Family SCOLOPENDRELLIDAE

KEY TO AUSTRALIAN GENERA OF SCOLOPENDRELLIDAE

First pair of legs consisting of 3 joints and being less than half as long as following pair ..... *Scolopendrellopsis* Bagnall

First pair of legs vestigial, represented only by small protuberances ..... *Symphylella* Silvestri

Genus SCOLOPENDRELLOPSIS Bagnall

SCOLOPENDRELLOPSIS EUCALYPTICA, sp. nov.

(Fig. 1)

Holotype.—One of the adult specimens from Gnangara, W.A.

Length.—Holotype, 2·07 mm. The length of the adult specimens ranges from 1·37–2·07 mm, with an average of 1·68 mm.

Head.—Head 1·4 times longer than broad with broadest part a little behind the middle. Points of articulation of mandibles very weakly developed; lateral margins of head evenly rounded. Length of central rod about half that of head. Rod interrupted a little before its mid-point, the anterior and median lateral branches lacking; on a level with the postantennal organs the rod becomes more and more indistinct and disappears. Dorsal surface of head rather evenly covered with straight, short setae. Diameter of postantennal organs not reaching three-quarters of the greatest diameter of 3rd antennal segment.

Antennae.—Antennae complete in the holotype, each comprising 18 segments of which the distal ones are not fully developed. In addition to these 18 there is also a short weakly developed segment, completely lacking in setae, at each antennal base. First segment of normal form and size, with 5 setae inserted on inner dorsal, inner, and ventral sides, one of the inner setae being the longest. Second segment with the primary whorl more completely set with setae on all sides, the longest being the inner dorsal one which length is about one-third of the greatest diameter of the segment. Setae of proximal segments longer than those towards the apex: about 50% longer in the case of the inner side. Proximal and middle parts of antennae have only primary whorls of setae on each segment, 2nd whorl beginning on 12th segment. No small circular sensory organs have been found and bladder-shaped organs (vide Scheller 1952, pp. 2 and 5) developed only on some distal segments. Apical segments thinner and shorter than preceding ones, their setae very short and fragile. No large forked organs distinguishable.

Terga.—First tergum rudimentary, with 6 setae, in two groups of 3; triangular posterior processes absent. The 13 terga having triangular processes vary considerably in form and size. Second tergum complete; distance between processes, measured along posterior margin of tergum, nearly 1·5 times as long as the length of the processes. Third tergum with distance between processes about as long as their length. On 4th tergum this ratio approximately agrees with that of 2nd tergum. Triangular processes prominent without end-swelling. They bear all an apical seta. No setae inserted between this and inner basal seta. Anterolateral setae of 2nd tergum short, being about one-third the length of

triangular processes. All marginal setae of nearly the same length. The number of marginal setae on different terga varies: 5 on 2nd, 6 on 3rd, and 6 on 4th tergum on the holotype but a slight variation is observable in other specimens. Posterior margin of terga bears setae between the triangular processes, 3 (on other specimens normally 2) on 2nd tergum and 2 on 3rd. Surface of terga sparsely set with straight setae of medium size. Form and chaetotaxy of head and first 4 terga is seen in Figure 1.

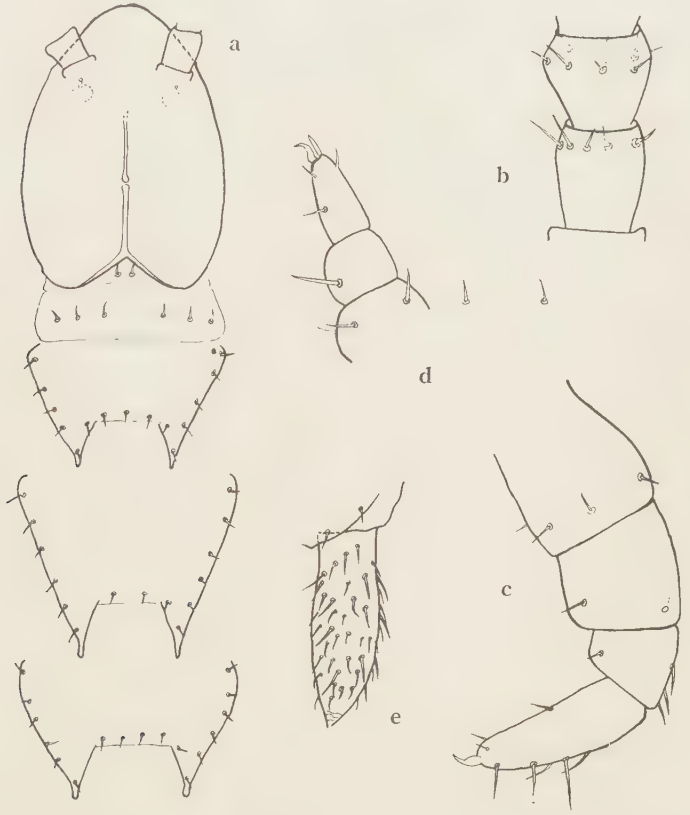

Fig. 1.—*Scolopendrellopsis eucalyptica,* sp. nov.: *a,* head and first 4 terga (only marginal setae of terga are drawn); *b,* first 2 antennal segments of right antenna, from above; *c,* 12th leg; *d,* 1st leg, from behind; *e,* right cercus, from above.

Legs.—All pairs well developed except first one which is reduced. Twelfth pair of legs covered with a short delicate pubescence. Tarsus of this pair short, being only 2·3 times longer than wide, and set with 5 setae of normal size on distal and middle part of outer side. Three of them are prominent and the remaining 2 are depressed. The longest of the protruding setae are about as long as greatest width of tarsus and a little longer than length of tibia. On inner side of tarsus there are a few short setae. Tibia as long as wide with at least 3 setae on upper surface. Femur a little shorter than wide. Anterior claw moderately slender with

a fairly robust base. Length of the more slender and more curved posterior claw about two-thirds the length of anterior claw. First pair of legs not fully developed consisting of only 3 joints. Its length about one-third the length of 2nd pair and longer than length of tarsus of 12th pair of legs. Femur and tibia about as wide as long while tarsus is about twice as long as wide, terminating in 2 claws, the anterior of which is a little longer than the posterior. Tarsus with at least 3 setae, 2 of which inserted on hind surface and 1 distally on anterior surface. Tibia with 1 fairly long seta on hind surface.

Cerci.—Cerci nearly cylindrical about 3·2 times longer than wide. They are widest at about the middle, and grow narrower towards the base. The number of setae large and length of setae varies considerably. On dorsal side there are 2 rows of large setae and such setae also occur on outer and ventral sides. The longest of them is one-half as long as greatest width of cercus. Remaining setae short, about one-half the length of longer setae, and they are depressed, most often also curved. Terminal area very short and faces outwards-downwards. Apical setae missing in all adult specimens.

Affinities.—The genus *Scolopendrellopsis* is very poor in species. The hitherto known species are *microcolpa,* described by Muhr (1881) from southern Italy and redescribed by Hansen (1903), and a species *sensiferis* described by Hilton (1931) from California. The latter, however, is very incompletely described, so incompletely that its identification seems to be impossible. That is why I here take into consideration only the European species for which we have access to Hansen's excellent description. There are many characteristics which separate *microcolpa* and *eucalyptica.* The most remarkable are the shape of the central rod system of the head, the setae of the antennae, the form of the 2nd tergum, the chaetotaxy of the triangular processes of the terga, the proportions of the tarsus of the last pair of legs, and the number and arrangement of the setae of the first pair of legs. The last-mentioned character Hansen did not discuss but Aubry and Masson (1952) have figured a leg of the first pair from *microcolpa* (p. 231).

Material examined.—19 specimens.

Distribution.—WESTERN AUSTRALIA: Stirling Ranges, 310 miles S. of Perth, Mt. Toolbrunup, in *Eucalyptus sp.* leaf litter and moss, 12.vi.1953, 4 ad.,* 1 juv. 11, 1 juv. 10, 1 juv. 9, 3 juv. 8; the same locality, in *Eucalyptus sp.* leaf litter, 12.vi.1953, 1 juv. 10, 1 juv. 8; Gnangara, 16 miles NE. of Perth, in *E. calophylla* leaf litter, 5.viii.1953, 5 ad.; the same locality and biotope, 27.viii.1953, 1 ad.; Kimberley Research Station, 62 miles E. of Wyndham, in *Adansonia gregorii* leaf litter, Feb.1954, 1 juv. stage?

Genus SYMPHYLELLA Silvestri

KEY TO AUSTRALIAN SPECIES OF THE GENUS SYMPHYLELLA

1. Mediotergal apodeme with well-marked frontal branches; cerci about 1/15 the length of the body ..... *bornemisszai,* sp. nov.

   Mediotergal apodeme without frontal branches; cerci shorter than 1/25 the length of the body 2

* Abbreviations: ad, a specimen with the maximum number of legs; juv. ..., a juvenile specimen with the number of pairs of legs indicated. These numbers include the rudimentary first pair of legs in *Symphylella.*

2. Cerci of normal shape, about 3 times longer than wide 3
   Cerci short, cylindrical, only about twice longer than wide .....*cylindrica*, sp. nov.
3. First antennal segment not half as long as following segment; tips of triangular processes of terga thin and lengthened .....*australiensis*, sp. nov.
   First antennal segment about as long as following segment; tips of triangular processes of terga of normal shape .....*tenuis*, sp. nov.

Symphylella cylindrica, sp. nov.

(Fig. 2)

Holotype.—Adult specimen from Gnangara, W.A.

Length.—1·65 mm.

Head.—Head only a little longer than broad with broadest part just behind the middle, on a level with the rounded points of articulation of mandibles. Central rod visible only in hind half of head. It is divided into two parts of equal length by a distinct interruption. Anterior and median lateral branches lacking and at middle of head the rod becomes more and more indistinct before it disappears. Dorsal surface of head sparsely covered with straight, short setae. Diameter of postantennal organs not reaching one-half of the greatest diameter of 3rd antennal segment.

Antennae.—Both antennae comprise 15 segments of which the distal ones are not fully developed. In addition to these 15 segments there is also a short, weakly developed segment at each antennal base. It completely lacks setae. First segment has only 1 whorl of setae, probably containing 6. The longest seta being the dorsal inner one. Its length is one-third of the greatest diameter of the segment. Setae of proximal segments longer than those towards apex. Proximal part of antennae has only 1 whorl of setae on each segment and the 2nd whorl, which occurs in the distal parts, is very inconsiderable and incomplete too. In the whorl of primary setae there are small circular sensory organs on 6th–13th segments. On each of these segments there is only one such organ inserted on dorsal inner surface. Another type of small, probably sensory organs is to be found, the bladder-shaped organs. They begin on the 12th segment. Terminal segment very short and seems to be without larger setae.

Terga.—First tergum rudimentary, triangular posterior processes absent and it bears 6 setae in two groups of 3. The 13 terga having triangular processes vary considerably in form and size. Second tergum complete; distance between processes, measured along posterior margin of tergum, nearly the same as length of processes. Third tergum with distance between processes 1·5 times as long as their length. On 4th tergum this ratio is 2. Triangular processes prominent without end-swellings. They all bear an apical seta. No setae inserted between this and inner basal seta. Anterolateral setae of 2nd tergum nearly three-quarters the length of processes. Anterolateral setae of nearly the same size throughout but a certain variation of the length can be observed in the marginal setae found between anterolateral and apical setae. Number of marginal setae on different terga varies. On the holotype there are 4 on 2nd, 5 on 3rd, and 4 on 4th tergum. No setae

can be seen on posterior margin between processes. Surface of terga sparsely set with straight setae of medium size. Form and chaetotaxy of head and first 4 terga is seen in Figure 2.

Legs.—All pairs well developed except the first one which is reduced to 2 very minute knobs. Twelfth pair of legs covered with a short delicate pubescence. Tarsus of this pair cylindrical and remarkably short and thick, only twice as long as wide and set with 4 fairly short setae on distal part of outer side. The longest of them only a little longer than one-half the width of the joint and about one-third

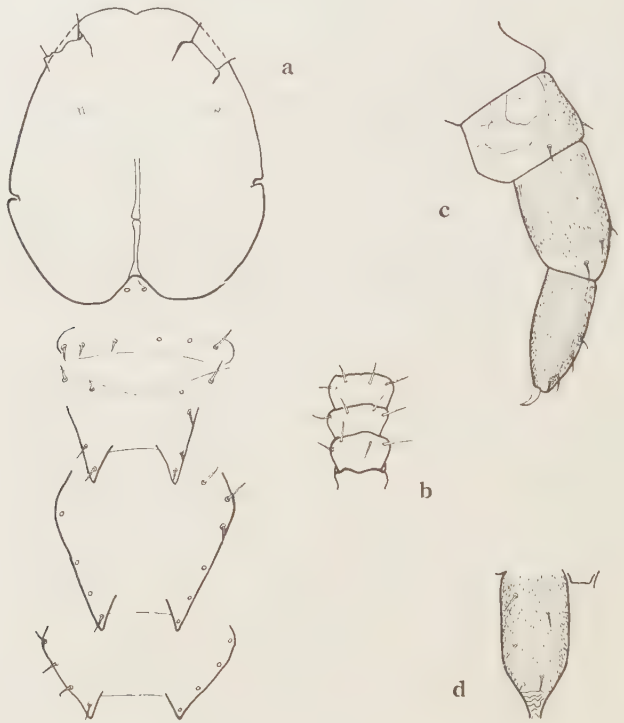

Fig. 2.—*Symphylella cylindrica,* sp. nov.: *a,* head and first 4 terga (only marginal setae of terga are drawn); *b,* basal part of left antenna, from above; *c,* 12th leg, from in front; *d,* right cercus, from below.

the length of the tibia. Last-mentioned joint 1·3 times longer than wide and longest seta of upper surface distinctly shorter than half the width of the joint. Femur nearly 1·5 times wider than long with pubescence only on outer surface.

Cerci.—Cerci cylindrical, short and thick, only a little more than twice as long as wide, not reaching 1/30 of the length of the animal. They are clothed with a fine pubescence and set with a few fairly short setae. Ventral surface with about 6 setae; fewer on dorsal surface. Apical setae lost. Terminal areas short, about one-third the width of cerci.

Affinities.—*S. cylindrica* resembles in the chaetotaxy of the triangular processes of the terga *S. elongata* Scheller from Europe, *S. maorica* Adam & Burtel from New Zealand, *S. foucquei* Jupeau from Madagascar and Réunion, *S. hintoni* Edwards from Europe, and *Symphylella* sp. from Lokobe, Madagascar, described by Rochaix (1956). However, it is easy to separate these latter species from the one described above by a number of good characters, especially in the shape and chaetotaxy of the cerci and the 12th pair of legs.

Material examined.—5 specimens.

Distribution.—WESTERN AUSTRALIA: Gnangara, 16 miles NE. of Perth, in *Banksia* sp. litter, 5.viii.1953, 1 ad., 4 juv. 11.

SYMPHYLELLA AUSTRALIENSIS, sp. nov.

(Fig. 3)

Holotype.—An adult specimen from Gnangara, W.A., 5.viii.1953.

Length.—1·85 mm. The length of adult specimens ranges from 1·57–2·27 mm with an average of 1·83 mm.

Head.—Head 1·3 times longer than broad with broadest part a little behind the middle. Points of articulation of mandibles do not stand out and for that reason lateral margins of head are evenly rounded. Central rod distinct only behind middle of head. The part of the rod which lies before the interruption point 1·5 times longer than hindpart, anterior and median lateral branches lacking, and at middle of head the rod becomes more and more indistinct before it disappears. Dorsal surface of head rather evenly covered with straight, short setae. Diameter of postantennal organs not reaching one-half the greatest diameter of 3rd antennal segment.

Antennae.—Major part of both antennae lost or broken in nearly all specimens. However, the number of segments seems to show a very low variation. In the few cases where the antennae are undamaged in adult animals, there are between 16 and 19 segments. Apical segment not fully developed. The short, incomplete, basal segment lacks setae. First segment weakly developed too, very short and set with setae only on inner dorsal and inner sides. Normally there are 3 setae on this segment, 2 inner dorsal (or seldom dorsal) and one inner lateral. This segment not one-half as long as following segment. Second segment with primary whorl much more complete with setae on all sides, altogether 6 in number, the longest of them being the inner dorsal one with its length being at least one-third of the greatest diameter of the segment. Setae of proximal segments longer than those towards the apex: nearly 50% longer in the case of the inner side. Proximal half of antennae has only primary whorls of setae on each segment, 2nd whorl beginning on the ventral side of 11th segment. Primary whorl with small circular sensory organs on inner part of dorsal side of the segments from 11th to 16th inclusive (one paratype specimen with 18 antennal segments). On each of these segments there is only one such organ. Bladder-shaped organs occur only on the distal quarter of the antennae. Apical segments thinner and much shorter than preceding ones, their setae being very short, about half the length of setae of proximal part of antennae.

Terga.—First tergum rudimentary bearing 6 setae, in two groups of 3; triangular posterior processes absent. The 13 terga having triangular processes vary considerably in form and size. Second tergum complete; distance between processes, measured along posterior margin of tergum, nearly the same as the length of processes. Third tergum with distance between processes about twice as long as length of processes. Fourth tergum with this ratio nearly 3. Triangular processes throughout not thickening towards their tips. They bear all an apical seta. No

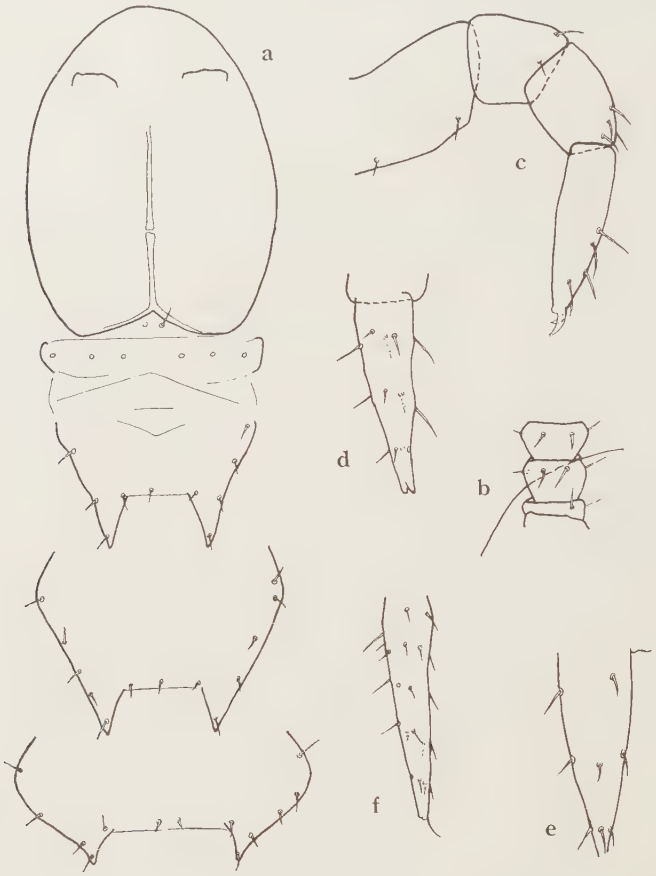

Fig. 3.—*Symphylella australiensis*, sp. nov.: *a*, head and first 4 terga (only marginal setae of terga are drawn); *b*, basal part of left antenna, from above; *c*, 12th leg; *d*, left cercus, lateral view; *e*, right cercus, from below; *f*, left cercus of an adult specimen from *Eucalyptus* sp. leaf litter at Mt. Toolbrunup, lateral view.

setae inserted between this and inner basal seta. Anterolateral setae of 2nd tergum about three-quarters the length of triangular processes. Anterolateral setae are of nearly the same size throughout but a certain variation in the length can be observed in the marginal setae found between anterolateral and apical setae. The number of marginal setae on different terga varies, 4 on 2nd, 5 on 3rd, and 4–5 on

4th tergum. Posterior margin of terga has setae between the triangular processes, one on 2nd tergum, one on 3rd, 2 on 4th and 5th. Surface of terga sparsely set with straight setae of medium size. Form and chaetotaxy of head and first 4 terga is seen in Figure 3.

Legs.—All pairs well developed except the first one which is reduced to 2 small knobs each with 1 seta of the same length as the knob itself. Twelfth pair of legs covered with a short delicate pubescence. Tarsus of this pair cylindrical, rather slender, not quite 3·5 times longer than wide and set with 5 setae on distal and middle part of outer side; the longest of them nearly as long as the greatest width of tarsus and nearly half the length of tibia. Last-mentioned joint somewhat more than 1·5 times longer than wide and the longest seta of upper surface half as long as the greatest width of tarsus. Femur about as wide as long.

Cerci.—Cerci conical, three times longer than wide with dorsal side straight. Their length smaller than 1/25 of the length of the animal. They are set with straight setae of which the longest are at least half the greatest width of cerci. Setae arranged in 4 rows, one dorsal, one ventral, and two lateral; they are also arranged in 3 distinct whorls. Apical setae lost but in an adult paratype specimen their length being three-fifths of the greatest width of cercus.

Variation.—Some specimens of this species, both adults and juveniles, present some differences from the holotype. Thus the ratio between the posterior margin of the 4th tergum and the length of its triangular processes ranges between 2·5 and 3. The number of posterior marginal setae also shows a slight variation. On the 2nd tergum there is most often one such seta (it is seldom lacking), on the 3rd tergum also there is normally one, seldom none, and on the 4th there can be 1–3 setae, however, there are most often 2. In one case there were 3 postero-marginal setae instead of the normal 2 on the 5th tergum. The number of marginal setae also varies slightly and this variation can often be observed between the two sides of the same tergum. The cerci are in some specimens proportionately longer than in the holotype and their setae are more numerous (Fig. 3*f*). Sometimes there are up to 5 whorls of setae instead of 3. Nevertheless, these slightly different specimens may belong to the same species as the one described above. In the greater part, in my opinion, the differences depend on the normal variation caused by the differences in age and body size. The most remarkable deviation from the normal specimens is seen in those that have the cerci conspicuously longer in proportion and with more numerous setae.

Affinities.—On the form and chaetotaxy of the terga this species is closely allied to *S. cylindrica*, *S. elongata* Scheller, *S. maorica* Adam & Burtel, *S. foucquei* Jupeau, *S. hintoni* Edwards, and Rochaix's *Symphylella* sp. from Lokobe, Madagascar. However, it can be distinguished from these species without difficulty especially by the chaetotaxy of the cerci.

Material examined.—132 specimens.

Distribution.—WESTERN AUSTRALIA: Stirling Ranges, 310 miles S. of Perth, Mt. Toolbrunup, in *Eucalyptus* sp. leaf litter and moss, 12.vi.1953, 8 ad., 7 juv. 11, 5 juv. 10, 16 juv. 9, 1 juv. 8; the same place, in *Daviesia* sp. leaf litter, 12.vi.1953, 1 juv. 11, 1 juv. 10; the same place in moss, 12.vi.1953, 2 juv. 11, 1 juv. 9; the same place, in leaf litter of

Eucalyptus sp. and *Melaleuca* sp., 12.vi.1953, 1 juv. 11, 1 juv. 10, 1 juv. stage?; the same place, in *Eucalyptus* sp. leaf litter, 12.vi.1953, 3 ad., 2 juv. 11, 2 juv. 10, 6 juv. 9, 1 juv. 8; Gnangara, 16 miles NE. of Perth, in *Banksia menziesii* leaf litter, 27.viii.1953, 1 ad., 1 juv. 11, 1 juv. 10; the same place, in *Eucalyptus calophylla* leaf litter, 5.viii.1953, 25 ad., 25 juv. 11, 2 juv. 9, 23.ix.1953, 2 ad., 1 juv. 11, 1 juv. 10; the same place and biotope, 28.x.1953, 7 ad., 7 juv. 8.

Symphylella bornemisszai, sp. nov.

(Fig. 4)

Holotype.—One of the adult specimens from Gnangara, W.A., *Eucalyptus calophylla* leaf litter, 27.viii.1953.

Length.—1·72 mm. The length of adult specimens ranges from 0·90 to 1·93 mm, with an average of 1·45.

Head.—Head 1·25 times longer than broad with broadest part a little behind the middle. Lateral margins flatly rounded, with points of articulation of mandibles nearly concealed. Central rod well developed with a sharp transverse suture at middle point of head. It has no vestige of lateral branches, but frontal branches strongly developed. The part of the rod which lies between forking point of anterior branches and interruption as long as the part behind interruption point. Dorsal surface of head rather sparsely covered with straight, short setae. Diameter of postantennal organs half as long as diameter of 3rd antennal segment.

Antennae.—Major part of both antennae lost or broken in nearly all specimens. Holotype with 16 segments in each antenna. All other specimens with complete antennae also have 16 segments except one which has 20 segments in both antennae. Apical segment not fully developed. The short and incomplete basal segment lacks setae. First segment of normal length but about one-sixth thinner than 2nd segment. It bears at least 5 setae, one on the inner side being the longest: one-third the diameter of the segment. It is only a little longer than the other setae of this segment. Setae of proximal segments longer than those towards the apex. Proximal half of antennae has only the primary whorl of setae on each segment, 2nd whorl beginning on ventral side of 10th–12th segments. In the primary whorl there are small circular sensory organs on inner parts of dorsal side of the segments from 10th to 15th inclusive. Each of these segments with only one such organ except 14th segment of both antennae where the number is 2. Bladder-shaped organs occur only in the distal part of the antennae. Apical segments thinner and much shorter than preceding ones and their setae rather short. A very small forked organ inserted distally.

Terga.—First tergum rudimentary with 6 setae, in two groups of 3; triangular posterior processes absent. The 13 terga having triangular processes vary considerably in form and size. Second tergum complete; distance between processes, measured along posterior margin of tergum, twice as long as processes. Third tergum with distance between processes as long as their length, while in 4th tergum the ratio is again 2. Triangular processes, which are all prominent, do not thicken towards their tips. They all bear an apical seta. No setae inserted between this and inner basal seta. Anterolateral setae of 2nd tergum about one-half the

length of processes of same tergum. Anterolateral setae of nearly the same size throughout which seems also to be valid for remaining marginal setae. Number of marginal setae between anterolateral and apical setae varies. Including them there are 5 marginal setae on 2nd tergum, 6 on 3rd, and 5 on 4th. Posterior margin of terga with setae inserted between processes, 3 on 2nd tergum, 2 on 3rd, and 4 on 4th. Surface of terga set with straight setae of medium size. Form and chaetotaxy of head and first 4 terga is seen in Figure 4.

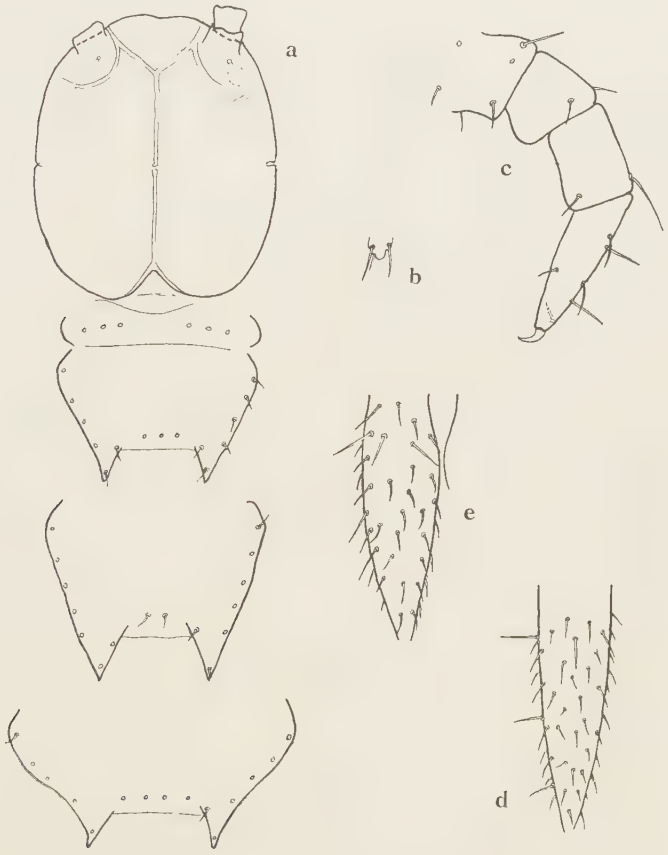

Fig. 4.—*Symphylella bornemisszai*, sp. nov.: *a*, head and first 4 terga (only marginal setae of terga are drawn); *b*, first leg; *c*, 12th leg, from in front; *d*, left cercus, lateral view; *e*, left cercus, from above.

Legs.—All pairs well developed except the first one which is reduced to 2 small ovoid knobs with a narrowing base. They are each set with 3 protruding setae at least as long as the knob. Tarsus of last pair of legs cylindrical, and rather slender, about 3 times longer than wide, and set with 5 setae, of which 3 are protruding and 2 are depressed and directed downwards. The longest of the 3 straight and outstanding setae nearly as long as the greatest width of tarsus and

somewhat longer than half the length of tibia. Last-mentioned joint 1·4 times longer than wide and longest seta of upper surface as long as the greatest width of tibia. Femur a little wider than long.

Cerci.—Cerci conical, between 3 and nearly 3·5 times longer than wide with dorsal side straight. They are proportionately long, about 1/14 of the length of the body. Cerci set with a fairly large number of setae, major part of which being rather short and often also depressed, while some being strongly protruding in all directions. Protruding setae always longer than the others and sometimes half as long as width of cerci. Terminal area very small. Apical setae lost.

Variation.—Some of the specimens show a slight variation in the number of posterior marginal setae of the terga. Often there are only 2 setae there on the 2nd tergum and exceptionally 2 or 3 on the 4th. There is also often a certain variation in the proportions of the cerci.

Affinities.—The chaetotaxy of the terga is rather similar to that of the preceding species *S. australiensis*, but *S. bornemisszai* is easily distinguished from it and from the other species with the same chaetotaxy of the terga (*S. elongata*, *S. maorica*, *S. foucquei*, *S. hintoni*, *S. cylindrica*, and Rochaix's *Symphylella* sp. from Lokobe, Madagascar) by a number of excellent characters. The central rod of the head, the 12th pair of legs, and the cerci show some good dissimilarities.

Material examined.—57 specimens.

Distribution.—WESTERN AUSTRALIA: Gnangara, 16 miles NE. of Perth, in *Banksia menziesii* leaf litter, 12.vi.1953, 2 ad., 1 juv. 11; the same place and biotope, 27.viii.1953, 1 juv. 11, 1 juv. 10; the same place, in *Eucalyptus calophylla* leaf litter, 5.viii.1953, 7 ad., 13 juv. 11, 3 juv. 10, 3 juv. 9; the same place and biotope, 27.viii.1953, 4 ad., 4 juv. 11, 1 juv. 9; the same place and biotope, 23.ix.1953, 3 ad.; the same place and biotope, 28.x.1953, 4 ad., 1 juv. 11, 9 juv. 8.

SYMPHYLELLA TENUIS, sp. nov.

(Fig. 5)

Holotype.—One of the adult specimens from Kimberley Research Station, W.A., Feb. 1954.

Length.—The holotype measures 1·42 mm, but some of the other adult specimens are up to 1·98 mm and one measures 1·41 mm.

Head.—Head 1·3 times longer than broad with broadest part at the middle. Lateral margins flatly rounded with points of articulation of mandibles nearly concealed. Central rod visible only in hind half of head. It is divided into two parts of equal length by an interruption. Anterior and median lateral branches lacking and a little behind mid-point of head the rod disappears slowly. Dorsal surface of head sparsely covered with straight, short setae. Diameter of postantennal organs reaches nearly three-quarters of the greatest diameter of 3rd antennal segment.

Antennae.—Major part of both antennae lost or broken in nearly all specimens. Holotype with only right antenna complete, comprising 14 segments. Remaining specimens with 1 or 2 undamaged antennae have always 17 or 21 antennal segments. Apical segment not fully developed. The short and incomplete

basal segment has no setae. First segment is of normal length, but a little thinner than 2nd segment. It has only the primary whorl consisting of 6 setae, 2 ventral, 2 inner lateral, 1 dorsal, and 1 outer lateral; longest setae being the inner ones, which are scarcely one-half of the greatest diameter of the segment. Distal part of antennae with much shorter setae than proximal part. Second whorl has not been examined. In the whorl of primary setae there are small circular sensory organs only on 11th–12th segments, one organ on each segment. No bladder-shaped organs visible. Apical segment thinner and much shorter than preceding ones and its setae very short.

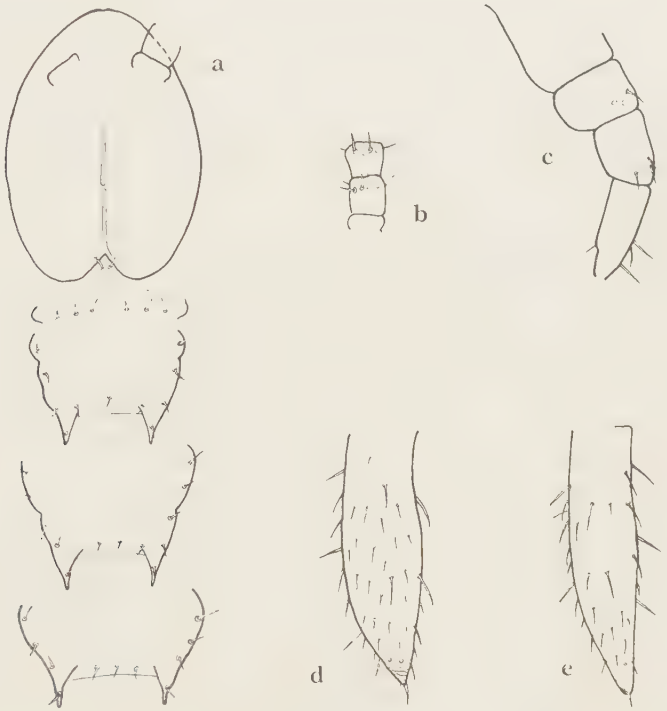

Fig. 5.—*Symphylella tenuis*, sp. nov.: *a*, head and first 4 terga (only marginal setae of terga are drawn); *b*, basal part of right antenna, from above; *c*, 12th leg; *d*, left cercus, from above; *e*, left cercus, lateral view.

Terga.—First tergum rudimentary, with 6 setae, in two groups of 3; triangular posterior processes absent. The 13 terga having triangular processes vary considerably in form and size. Longer terga, at least of anterior part of the body, with lateral margins slightly concave as in the genus *Symphylellopsis*. Tips of triangular processes very characteristic: lengthened, thin, and straight. Second tergum complete; distance between processes, measured along posterior margin of tergum, nearly 1·5 times longer than processes. Third tergum with distance between processes as long as their length, while on 4th tergum this ratio is nearly 2. The processes bear all an apical seta. No setae inserted between this and inner

basal seta. Anterolateral setae of 2nd tergum about half as long as processes of same tergum. The anterolateral setae are the longest ones and of nearly the same size throughout. Number of marginal setae between anterolateral and apical setae varies. Including end-setae there are 4 marginal setae on 2nd tergum, 5 on 3rd, and 4 on 4th tergum. Posterior margin of terga with setae between processes, 1 (in most adult specimens 2) on 2nd tergum, 2 on 3rd, and 3 on 4th. Surface of terga sparsely set with straight setae of medium size. Form and chaetotaxy of head and first 4 terga is seen in Figure 5.

Legs.—All pairs well developed except the first, which is reduced to 2 small nearly cylindrical knobs, each with 3 protruding setae, of which at least 2 are longer than the knob itself. Tarsus of last pair of legs cylindrical and rather slender, not quite 3 times longer than wide, with only 4 setae on outer side. It has not been possible to study the setae in detail but the longest of them seems not to be as long as the greatest width of the joint. Tibia 1·25 times longer than wide, with 3 setae on distal part of upper surface. Femur a little wider than long. Last pair of legs very short, about as long as cerci.

Cerci.—Cerci of the same length as 12th pair of legs, 3 times longer than wide (in some other specimens a little more), and their length not reaching 1/25 of the length of the body. Dorsal side straight, ventral and outer lateral sides strongly curved. Cerci with a fairly large number of setae inserted mainly on outer three-quarters of the organs. The greater number of them are short, curved, and depressed while some are strongly protruding, especially 2–3 on dorsal side and 3–4 on outer lateral side. Protruding setae always longer than the others and sometimes longer than half the width of the cerci. Terminal area small, looking downwards and outwards. The larger one of apical setae lost.

Affinities.—*S. tenuis* belongs to the same group of species as the preceding ones. It has many characters in common with them but it differs above all with regard to the form of the terga, the exceedingly low number of antennal segments bearing circular sensory organs, the chaetotaxy of the cerci, and the length of the 12th pair of legs in proportion to the length of the cerci.

Material examined.—17 specimens.

Distribution.—WESTERN AUSTRALIA: Kimberley Research Station, 62 miles E. of Wyndham, in *Eucalyptus* sp. leaf litter, Feb. 1954, 4 ad., 7 juv. 10, 4 juv. 9, 1 juv. 8, 1 stage?

SYMPHYLELLA spp.

Some inadequate material of two additional undescribed species of *Symphylella* has been examined.

Species A

Material examined.—3 specimens, labelled *Symphylella* sp. A.

Distribution.—WESTERN AUSTRALIA: Kimberley Research Station, 62 miles E. of Wyndham, in leaf litter of *Bauhenia* sp. and *Terminalis* sp., Feb. 1954, 1 ad., 1 juv. 11, 1 juv. 9.

An exact placing of these three specimens has not been possible as the single adult animal has neither antennae nor cerci. Moreover, the setae of the

legs are lost for the greater part. However, they are certainly allied to *S. vulgaris* (Hansen) but are not identical with it even if the antennae and the cerci should prove to agree. The anterior branches of the central rod are well developed in *vulgaris* while it is not so here. The triangular processes of the 3rd tergum are proportionately much longer than in *vulgaris* and the distance between them in relation to the length of the processes is only a little over 1 in the Wyndham specimens while it is at least 2 in *vulgaris*.

Species B

Material examined.—1 specimen, labelled *Symphylella* sp. B.

Distribution.—NORTH QUEENSLAND: Hinchinbrook I., *c.* 75 miles N. of Townsville, in a decaying log in tropical rain-forest, Sept. 1956, 1 juv. 10.

It is impossible to reach a perfect determination of this animal as it is not full-grown. However, as it deviates highly from the above described species it is evident that it is a member of *Symphylella* not before found in Australia.

Family **SCUTIGERELLIDAE**

KEY TO AUSTRALIAN GENERA OF SCUTIGERELLIDAE

Last tergum with deep posterior depression; all terga emarginate posteriorly; styli of posterior legs short ..... *Scolopendrelloides* Bagnall

Last tergum without deep posterior depression; first two terga convex posteriorly; styli of posterior legs large ..... *Hanseniella* Bagnall

Genus SCOLOPENDRELLOIDES Bagnall

In the genera and species of the order Symphyla, Hansen (1903) said that the Symphyla consists of one single family with the two genera, *Scutigerella* and *Scolopendrella*. This simple division is no longer valid. Hansen too was conscious that his two generic groups were very heterogeneous and foresaw the establishment of new families and genera by a subdivision of the two described genera. Hansen's expectation was well-grounded, as we now have two families containing altogether 14 genera. One of these genera, *Scolopendrelloides*, was split off from Hansen's *Scutigerella* by Bagnall (1913). After having established *Hanseniella* he divided it into two subgenera, *Hanseniella* s.s. and *Scolopendrelloides*, and diagnosed the latter as follows (p. 197):

"The last scutum with a deep postero-median depression. The exopods of posterior legs short, at most much shorter than the depth of tarsus. Some setae on the inner side of the proximal antennal joints nearly vertical to the longitudinal axis of the antennae and unusually long, the longest at least two and one-half times as long as the setae on the outer side."

All these features had been observed and pointed out by Hansen (1903, pp. 25, 26) but he did not value them as generic characters. Bagnall too was cautious, but now *Scolopendrelloides* has the status of genus (Edwards 1959*a*).*

* However, the last one of Bagnall's generic characters must be deleted (see *Remarks*, p. 158).

Scolopendrelloides bifida, sp. nov.

(Fig. 6)

Holotype.—One of the fully grown specimens from Kimberley Research Station, W.A. Feb. 1954.

Fig. 6.—*Scolopendrelloides bifida,* sp. nov.: *a,* first 4 terga (only marginal setae are drawn); *b,* base of right antenna, from above; *c,* lanceolate setae from the same antenna; *d,* 12th leg, from behind; *e,* stylus; *f,* posterior part of 15th tergum and right cercus, from above.

Length.—The holotype measures 2·15 mm and the second adult specimen 1·49 mm.

Head.—Head one-quarter longer than broad with lateral angle at point of articulation of mandible rounded. Central rod conspicuous but short and visible only in hind half of head. Its length from the very marked posterior end-swelling to disappearing point forwards about one-quarter the length of the head. It ends anteriorly before it passes one-quarter to one-third of the distance of the length of the head. No lateral branches have been found and in front the rod becomes more and more indistinct and disappears at the centre of the head. Dorsal surface of head densely covered with short, straight, evenly scattered setae. Postantennal organs globular, their diameter a little smaller than one-third of the greatest diameter of 3rd antennal segment.

Antennae.—Antennae of holotype badly damaged and consequently not suitable for study. Therefore, the following description of the antennae is based on observations on the smaller adult specimen, where some characters can be studied on the base and middle part of the right antenna. First segment with only 1 whorl of setae which is not fully developed as there are setae only on inner half of the segment, the longest of these setae being at least as long as half the greatest diameter of the segment. Second segment too with the single incomplete primary whorl. The most proximal part of the antennae has only 1 whorl of setae on each segment, secondary whorl beginning on inner part of 7th segment. Small 4-spined organs beginning on inner side of 3rd segment. From 6th segment onwards there is a little lanceolate seta inserted in primary whorl on inner part of dorsal side of each segment. This seta very short, only about one-fifth the length of longer primary setae.

Terga.—First tergum rudimentary bearing about 10 setae. Second tergum fully developed, with 2 posterior triangular processes twice as broad as long, and with 3 short straight setae on each inner margin. Distance between tips of processes twice as long as length of processes. Apical setae not longer than the others. Anterolateral setae broken. Number of marginal setae between apical and anterolateral ones is 7. This tergum fairly small, 1·5 times broader than long. On lateral margins behind anterolateral macrochaetae there is also another fairly large marginal seta inserted on each side. Third tergum larger and more than 1·5 times broader than long. Posterior triangular processes broader than on preceding tergum, between 2·5 and 3 times broader than long with 3 marginal setae on their inner sides. Number of lateral marginal setae larger than on 2nd tergum. Distance between tips of processes only a little more than twice as long as length of processes. Fourth tergum broad and short, at least twice as broad as long, with shorter and broader triangular processes with 4 inner marginal setae. This tergum has about 10 lateral marginal setae between anterolateral macrochaetae and apical setae. Anterolateral macrochaetae broken or lost, but, judging from unbroken setae and large areas of insertion, at least terga 2, 3, and 4 with such machrochaetae. It is probable that they also occur more posteriorly.

Posterior median cavity of 15th tergum U-shaped and about twice as long as broad. Anterior part a little overlapped. In the juvenile specimens the cavity is not fully developed as anterior overlapping is not found.

Legs.— Tarsus of 12th pair of legs rather short and strongly tapering towards distal end, 2·5 times longer than wide, with dorsal side nearly straight and ventral side more curved. On dorsal side there are 2 rows containing 3 and 2 setae respectively. These setae of nearly the same length, the longest of them as long as greatest diameter of the joint. On ventral side setae are numerous but short and they stand out perpendicularly and are rather evenly scattered over ventral surface. Length of these setae not one-quarter of the diameter of tarsus. Tibia between 1·3 and 1·5 times longer than wide, covered with setae on both dorsal and ventral sides. The longest one of these setae located on distal half of dorsal side, its length being nearly three-quarters of the greatest diameter of the joint.

First pair of legs consists of 4 joints. Tarsus nearly cylindrical and tapering towards distal end, being about 4 times longer than wide. Chaetotaxy of this pair of legs not available for study.

Claws small. Length of anterior claw of 12th pair of legs not reaching one-sixth of the length of tarsus.

Styli proportionately short, only 1·5–1·75 times longer than wide with rather a broad base, being much shorter than the diameter of tarsus. The long apical seta not as long as length of stylus.

Cerci.—Cerci a little more than 3 times longer than wide. Dorsal sides straight, ventral and inner lateral ones curved. There are a moderate number of medium-sized setae, the longest being the most distally located ones of dorsal surface. Their length is half the greatest width of cerci. They are one-third longer than proximal setae. Apical setae lost.

Remarks.—Contrary to the nearly related *Hanseniella,* the genus *Scolopendrelloides* seems to be very poor in species. Up till now only the two species *crassicornis* and *pauperata* are known. They were described by Hansen (1903) from Koh Chang I. in the Gulf of Siam. These species, however, have not been found again. Thus *S. bifida* is an interesting find. It is the third known species of the genus and it extends the known geographical distribution from south-eastern Asia to the Australian continent.

The above-diagnosed species differs from *crassicornis* and *pauperata* in that it has all antennal setae of the same length and direction as the vast majority of the species of the family. Because of this deviation it seems necessary to delete the third and last part of the generic diagnosis given by Bagnall. The relative length of the setae and their direction are often very doubtful characters within the Symphyla and such insignificant features of the structure of the body must not be used in generic diagnoses before a careful examination has been carried out.

Material examined.—4 specimens.

Distribution.—WESTERN AUSTRALIA: Kimberley Research Station, 62 miles E. of Wyndham, in litter of *Eucalyptus platyphylla* and *Pandanus,* Feb. 1954, 2 ad., 1 juv. 9, 1 juv. 8.

Genus HANSENIELLA Bagnall

Key to Australian Species of the Genus Hanseniella

1. Large anterolateral setae at least on terga 2, 3, 4, 6, 7, and 9; these setae longer than diameter of 1st antennal segment 2
   Large anterolateral setae only on terga 2 and 3; these setae not as long as diameter of 1st antennal segment ..... *silvicola*, sp. nov.
2. Third tergum more or less emarginate posteriorly 3
   Third tergum with posterior margin straight or slight convex 4
3. First rudimentary tergum with 2 setae; 3rd tergum only slightly emarginate ..... *indecisa* (Attems)
   First rudimentary tergum with 4 setae; 3rd tergum more emarginate ..... *similis*, sp. nov.
4. Third whorl of setae on antennae fully developed 5
   Third whorl of setae on antennae missing ..... *minor* Tiegs
5. Third whorl of setae on antennae begins on about 12th–13th segment 6
   Third whorl of setae on antennae begins on about 17th segment ..... *agilis* Tiegs
6. First rudimentary tergum with 2 setae; cerci wear 2–4 setae in each row ..... *lucifuga*, sp. nov.
   First rudimentary tergum with 4 setae; cerci wear 7–8 setae in each row ..... *armigera*, sp. nov.

Hanseniella indecisa (Attems)

Scutigerella indecisa Attems, 1911, pp. 165–7.

Attems (1911) reported for the first time the occurrence of the Symphyla in Australia when he described, however in some respects indistinct, *H. indecisa* under the name of *Scutigerella indecisa* from six south-western stations: Lion Hill, Guildford, Harvey, Brunswick, Boyanup, and Gooseberry Hill. From the same part of the continent the present author has examined seven adult specimens collected by Prof. T. Gislén in 1952 from two stations, and, on the basis of that material, improved Attem's original description (cf. Scheller 1954*a*, pp. 3–6). Outside the Australian continent *H. indecisa* has been reported from New Zealand only (Adam and Burtel 1956).

Hanseniella minor Tiegs

Hanseniella minor Tiegs, 1939, pp. 8–9.

This species has been found only in Australia but it does not occur in Bornemissza's collection. It was described from Belgrave, Marysville, and the Otway Ranges in Victoria by Tiegs in 1939. He found it under stones and in decaying vegetation, especially in well-decayed trunks of the tree-ferns *Alsophila* and *Dicksonia*.

Tiegs says (1939, p. 10) that *H. minor* seems to resemble most closely *H. subunguiculata* (Imms) from the Himalayas but he also pointed out some distinguishing characters of which the two last-mentioned, the form of the claws and the chaetotaxy of the cerci, are significant.

Hanseniella agilis Tiegs

Hanseniella agilis Tiegs, 1939, pp. 5–7.

According to Tiegs (1939) this species is common in Australia. He mentions three places in Victoria where he had obtained it: Belgrave, Marysville, and the Otway Ranges.

Of the eight animals the present author has studied one fully grown specimen is easy to place in *agilis* but the rest are in some respects not identical with Tiegs' material, judging from the description. Probably this depends on the presence of several stages of development. In reaching full development the adult animal passes through numerous changes connected with the moults.

Material examined.—8 specimens.

Distribution.—VICTORIA: Gunyah, 28 miles S. of Morwell, under bark of decaying pine stump, 25.ii.1957, 8 ad.

Hanseniella lucifuga, sp. nov.

(Fig. 7)

Holotype.—Adult specimen, Hinchinbrook I., N. Qld., Sept. 1956.

Length.—2·6 mm.

Head.—Head with nearly the same breadth as length, but is a little broader than long. Lateral angle at point of articulation of mandible rounded. Central rod conspicuous but short, visible only in hind half of head. Its length from posterior end-swelling to disappearing point forwards scarcely one-quarter the length of head. It ends before it passes one-quarter to one-third of the distance of the length of head. No lateral branches have been found, and in front the rod becomes more and more indistinct and disappears at centre of head. Dorsal surface of head densely covered with straight and evenly scattered setae. The longest one of the setae inserted in front of lateral angle only a little shorter than the greatest diameter of 1st antennal segment.

Antennae.—Left antenna with 23 and right with 22 segments. The only whorl of setae of 1st segment comprising 6 setae located on dorsal, inner, and ventral surfaces. The longest of them only a little more than half the greatest diameter of the segment. Second segment also with the single primary whorl only with its setae being rather evenly inserted around the segment. Setae of inner side of about the same length as the others. The most proximal parts of the antennae have only 1 whorl of setae on each segment, secondary whorl beginning on ventral side of 5th–7th segment. More distally on ventral side of 13th segment the 3rd whorl begins. It fuses the primary and secondary whorls so that it is very difficult to distinguish the 3 whorls. On distal part of antennae there are also smaller setae inserted in distal rings. Small 4-spined organs begin on 2nd segment. Terminal segment somewhat longer than wide, provided with a large number of mostly anteriorly directed setae all of nearly the same length. All prominent setae of this segment much shorter than those of the basal part of antennae. The latter at least twice longer than distal setae. A large 4-spined organ arises from a circular protuberance at the apex of terminal segment. Its length is only a little

more than one-third the length of the segment. All antennal segments with a delicate pubescence. Setae of inner side of proximal half of antennae not as prominent as the rest of setae except those of terminal segment.

Terga.—First tergum rudimentary with 2 setae. Second tergum complete with posterior margin convex and having distinct, lateral angles with large anterolateral setae. Between these long setae 21 setae of shorter but different lengths are found, the longest reaching more than one-third the length of anterolateral setae, while the shortest ones are about one-sixth of this length. Surface of tergum set with setae of medium size. This tergum about twice as broad

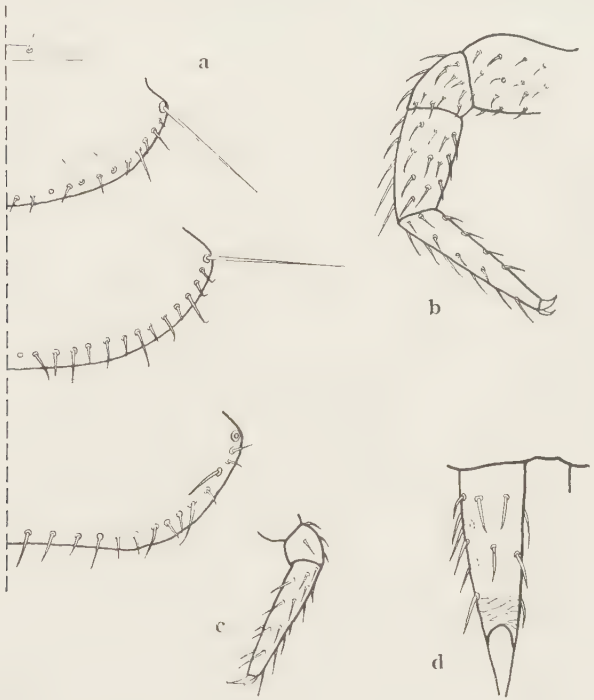

Fig. 7.—*Hanseniella lucifuga,* sp. nov.: *a,* first 4 terga (only marginal setae are drawn); *b,* 12th leg, from in front; *c,* first leg, from in front; *d,* right cercus, from above.

as long. Third tergum a little larger than second, being broader than head, and its posterior margin straighter and the number of setae between anterolateral setae greater, amounting to 28. Anterolateral setae nearly 3 times longer than diameter of 1st antennal segment. Most anterolateral macrochaetae missing behind 2nd and 3rd terga, but judging from large areas of insertion, it seems as if terga 2, 3, 4, 6, 7, and 9 all have such setae.

Legs.—Tarsus of 12th pair of legs slender at distal end, but widens towards upper part. It is 4 times longer than wide and has both dorsal and ventral sides straight and is covered with numerous setae arranged in rows lengthways. In

the dorsal row there are 6 setae, in the ventral 5. Most distal seta of dorsal row more prominent than the others and nearly two-thirds of the greatest diameter of the joint. All setae of nearly the same length. Tibia twice as long as wide, and, like tarsus, covered with setae on all sides, 5 in the dorsal row and 3 in the ventral. The longest one of these setae located on dorsal side near distal end and its length being nearly one-half the greatest diameter of the joint. Femur naked on dorsal side.

First pair of legs short with a nearly cylindrical tarsus strongly tapering towards the end. This joint about 4 times longer than wide with 6 setae in dorsal row and 4 in ventral. In all the rows distally located setae longer than the others. The longest seta, about as long as the diameter of the joint, is the most distally located in the dorsal row. Second joint with fully developed setae only on dorsal side, while 3rd joint has dorsal side naked and ventral surface set with several partly very long and partly short setae, the longest of them being nearly as long as the width of the joint.

First pair of legs with anterior claw very elongate, slender, and only very little curved, one-fifth the length of tarsus. It is about twice as long as posterior claw. Anterior claw of 12th pair of legs shows a high degree of similarity to that of first leg but is larger, one-fifth to one-quarter the length of tarsus and a little more curved.

Styli well developed, elongate bodies, 2·5–3 times longer than wide, decidedly shorter than diameter of tarsus, and densely covered with a short pubescence. The two apical setae of different lengths, the longer one being about half the length of stylus, the shorter about half the length of longer seta.

Cerci.—Cerci 3·7 times longer than wide and set with a fairly small number of setae of moderate length; the distal ones being a little longer than the proximal. There are 3 setae in one row on the inner side and 4 in the longest outer row. The longest seta is the most distally located one of the outer row and its length is 2/3 the greatest diameter of cercus. Apical setae lost.

Affinities.—The distribution of the macrochaetae is identical with that described in *H. caldaria* (Hansen), *echinata* Adam & Burtel, *milloti* Aubry & Masson, *orientalis* (Hansen), *plebeia* (Hansen), and *unguiculata* (Hansen) but this new species has some easily distinguishable separating characters in the form and the arrangement of the setae of the cerci.

Material examined.—1 specimen.

Distribution.—NORTH QUEENSLAND: Hinchinbrook I., *c*. 75 miles N. of Townsville, in a decaying log in tropical rain-forest, Sept. 1956, 1 ad.

HANSENIELLA SILVICOLA, sp. nov.

(Fig. 8)

Holotype.—One of the adult specimens from Baron Falls, N. Qld., Sept. 1956.

Length.—2·2 mm.

Head.—Head nearly as long as broad. Lateral angle at point of articulation of mandible rounded. Central rod conspicuous but short, visible only in hind half

of head. No lateral branches have been found, and in front the rod becomes more and more indistinct and disappears at centre of head. Dorsal surface of head densely covered with straight and evenly scattered setae. The longest one of the setae inserted in front of lateral angle scarcely three-quarters of the diameter of first antennal segment.

Antennae.—Antennae short; the left one broken at 15th segment but the right undamaged, comprising 16 segments. The only whorl of setae of 1st segment with 6–7 setae rather evenly scattered around the segment. Second segment too with only the single primary whorl comprising 7 setae. Setae of inner side of about the same length as the others. The most proximal parts of antennae bearing only 1 whorl of setae on each segment, secondary whorl beginning on dorsal side of 3rd segment and on ventral side on 3rd–5th segment. Further distally on ventral side of 8th–9th segment 3rd whorl begins. It fuses the primary and secondary

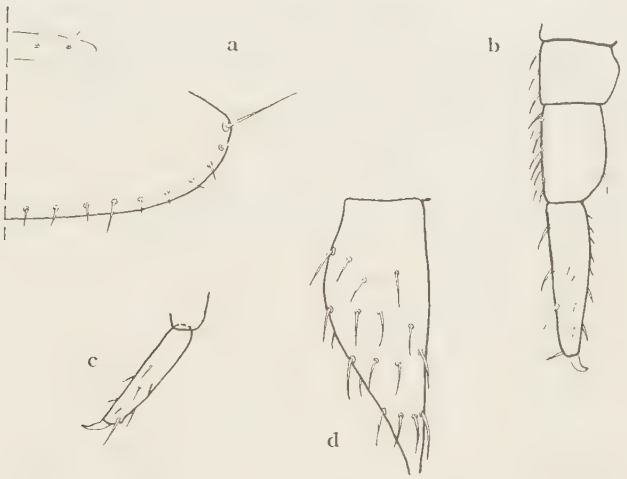

Fig. 8.—*Hanseniella silvicola*, sp. nov.: *a*, first 2 terga (only marginal setae are drawn); *b*, 12th leg, from in front; *c*, tarsus of first leg, from in front; *d*, left cercus, from above.

whorls so that it is very difficult to distinguish the 3 whorls. The distal part of antennae also bearing smaller setae inserted in distal rings. Small 4-spined organs, which are normally seen in the primary whorl of most *Hanseniella* species, absent here. Terminal segment somewhat longer than wide and provided with mostly anteriorly directed setae which are only a little longer than half the length of the setae of basal part of antennae. A relatively short 4-spined organ arises from a circular protuberance at the apex. Its length is only a little more than one-fifth of the length of the segment. Pubescence of antennal segments very fine. Setae of inner side of proximal half of antennae not as prominent as the rest.

Terga.—First tergum rudimentary with 4 setae. Second tergum complete with posterior margin convex and with distinct, lateral angles with short antero-lateral setae. Between these macrochaetae 19 setae are found; they are shorter and

of different lengths. Surface of tergum set with fairly short setae. This tergum about 3 times broader than long. Third tergum broader than head and also broader than preceding tergum; posterior margin nearly straight and number of setae between anterolateral macrochaetae larger than on 2nd tergum. Length of this tergum half the breadth. Anterolateral macrochaetae not as long as diameter of first antennal segment. They occur only on 2nd and 3rd terga. Fourth tergum with a slightly emarginate posterior border. This tergum nearly 3 times as broad as long with more than 30 setae between macrochaetae.

Legs.—Tarsus of 12th pair of legs relatively slender at distal end, but widens towards upper part. It is 3·5–4 times longer than wide and has dorsal side straight but ventral side with strongly widened basal part. In the dorsal row of tarsus there are 4 setae, in the ventral only 2 of normal size. The most distal seta of dorsal row more prominent than the others, its length being nearly three-quarters of the greatest width of the joint. On upper part of ventral side of tarsus there are setae, shorter than the ones of distal part of the joint but larger than pubescence hairs. Tibia 1·5 times as long as wide with numerous setae on its dorsal side; on ventral side only one seta of normal size. Longest seta of this joint located on dorsal side near distal end and its length is nearly half of the greatest diameter of the joint.

First pair of legs short with a nearly cylindrical tarsus strongly tapering towards distal end. This joint 5–5·5 times longer than wide, with 2–3 setae in the dorsal row and 2 in the ventral. Dorsal setae a little longer than ventral ones, the longest being somewhat longer than diameter of the joint.

Cerci.—Cerci 2·75 (in second specimen a little more than 3) times longer than wide, with dorsal side straight. They have 4 ventral and 2 dorsal rows of setae of moderate thickness, of which the distal ones are about 1·75 longer than the proximal. Longest setae being the most distally located ones and their length is somewhat more than half of the greatest diameter of the cercus. Apical setae lost.

Affinities.—This species is sharply separated from the other forms by the distribution and length of the anterolateral macrochaetae. It is also easily recognized from all other forms of the genus by the distribution of the setae of the tarsus of the last pair of legs.

Material examined.—3 specimens.

Distribution.—NORTH QUEENSLAND: Baron Falls, 20 miles NW. of Cairns, under decaying wood in tropical rain-forest, Sept. 1956, 2 ad., 1 juv. 8.

HANSENIELLA ARMIGERA, sp. nov.

(Fig. 9)

Holotype.—One of the adult specimens from Gunyah, Vic., 10.iii.1957.

Length.—A fairly large species with an average length of 5·2 mm. Holotype measures 4·8 mm.

Head.—Head with nearly the same breadth as length, but is a little longer than broad. Lateral angle at point of articulation of mandible marked but rounded. The short but conspicuous central rod visible only in hind half of head. Its length from posterior end-swelling to disappearing point forwards about one-quarter the length

of head. It ends posteriorly before it passes one-quarter to one-third of the distance of the length of head. No lateral branches have been found, and in front the rod becomes more and more indistinct and disappears at centre of head. Dorsal surface of head densely covered with straight and evenly scattered setae, the longest being the two inserted between antennae. Their length equal to greatest diameter of 1st antennal segment or shorter.

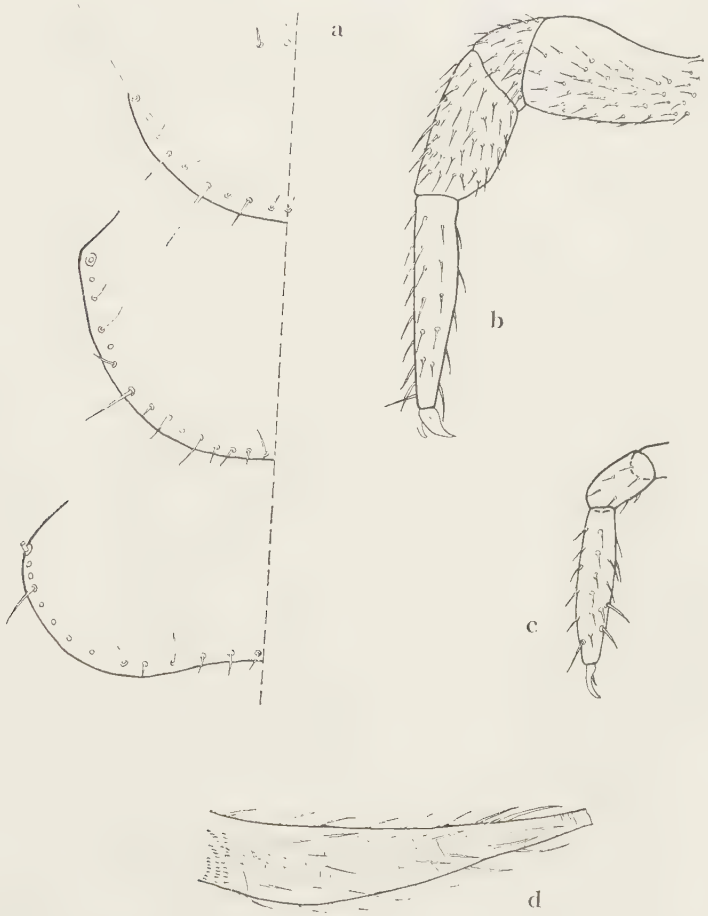

Fig. 9.—*Hanseniella armigera,* sp. nov.: *a,* first 4 terga (only marginal setae are drawn); *b,* 12th leg, from in front; *c,* first leg, from in front; *d,* left cercus, from outer side.

Antennae.—Major part of both antennae lost or broken, but the holotype has unbroken antennae, the left with 26 segments and the right with 22. First segment with only 1 whorl of 6 setae, 2 located on dorsal side, 2 on inner, and 2 on ventral side. They are all at least as long as half of the greatest diameter of 1st segment. Second segment too with a single primary whorl. Its setae rather evenly inserted around the segment. Setae of inner side of about the same length as the others. The most proximal parts of antennae with only 1 whorl of setae on each segment,

secondary whorl beginning on inner side of 4th–7th segment. Further distally on ventral side of 12th segment 3rd whorl begins. It fuses primary and secondary whorls so that it is very difficult to distinguish the 3 whorls. Small 4 spined organs begin on dorsal side of 2nd -3rd segment. Terminal segment nearly 1·5 times longer than wide and provided with a large number of mostly anteriorly-, outwards-, and upwards-directed setae. All prominent setae here not as long as half the length of setae of middle and proximal part of antennae. A large 4-spined organ arises from a circular protuberance at apex of terminal segment. Its length is one-quarter the length of the segment. All antennal segments bearing a delicate pubescence. Setae of proximal part of antennae not as prominent as following setae.

Terga.— First tergum rudimentary, bearing 4 setae. Second tergum fully developed with posterior margin convex. It has distinct lateral angles with large anterolateral macrochaetae, being directed outwards and forwards. Between these long setae 17–18 setae of shorter and different lengths are found, the longest reaching half the length of anterolateral macrochaetae, the shortest ones being 0·1–0·2 of this length. Surface of tergum set with setae of medium size. This tergum about twice as broad as long. Third tergum similar to second but a little larger and somewhat broader than head. Posterior margin straighter and number of setae between anterolateral ones greater here than on preceding tergum and amounts to 30; holotype bearing 25 which is the average number of adult specimens. Anterolateral setae twice as long as diameter of 1st antennal segment. Fourth tergum more than twice as broad as long with posterior margin rather emarginate. Most of the anterolateral macrochaetae missing behind 1st tergum, but judging from the occurrence of unbroken setae and large areas of insertion, it seems as if terga 2, 3, 4, 6, 7, and 9 all have such setae.

Legs.—Tarsus of 12th pair of legs slender at distal end but widens towards upper part, being 4·5–5·5 times longer than wide, with both dorsal and ventral side nearly straight. Setae arranged in rows lengthways. In dorsal row there are 6-7 setae, in ventral 4–5. The most distal seta of dorsal row more prominent than the others. Its length about 1·25 of the greatest width of the joint.

First pair of legs short with a nearly cylindrical tarsus strongly tapering towards the end. This joint is 3·25–4·5 times longer than wide, with usually 4 setae in dorsal row and 3 in the ventral. Distally located setae always longer than the others, the longest one, the most distally located in dorsal row, being as long as or somewhat longer than the greatest width of the joint. Second joint with setae mainly on dorsal side while 3rd joint with dorsal side naked and ventral surface set with several partly very long and partly short setae.

First pair of legs with an elongate anterior claw, very slender and very little curved, one-quarter the length of tarsus. It is one-third longer than posterior claw. Anterior claw of 12th pair of legs broader and more robust than that of 1st pair of legs. It is also larger and more curved, usually shorter than one-quarter the length of tarsus.

Styli well developed, elongate, nearly 4 times longer than wide, decidedly longer than width of tarsus and densely covered with a short pubescence. The 2 apical setae of different lengths, the longer one about half the length of stylus, the shorter about half the length of longer seta.

Cerci.—Cerci somewhat more than 4 times longer than wide with a large number of setae, those of the proximal two-thirds of cerci of moderate size, while the length increases more distally. Setae arranged in 7 rows with 7–8 setae in dorsal row. Longest setae being the most distally located ones and their length is three-quarters of the greatest width of the cercus. There are 2 apical setae, a median one and a lateral one, the former long, about one-quarter the length of cercus. The most proximal part of the organ covered with a very delicate pubescence which is absent distally.

Affinities.—This species resembles a large number of other species in the distribution of the macrochaetae of the terga, in this respect agreeing with *lucifuga* (p. 162).

Material examined.—23 specimens.

Distribution.—VICTORIA: Gunyah, 28 miles S. of Morwell, in a decaying, very moist *Eucalyptus* log in temperate rain-forest, 26.i.1957, 4 ad., 1 juv. 11, 3 juv. 10, 1 juv. 9; the same place and biotope, 27.i.1957, 8 ad., 3 juv. 10; the same place, under *Eucalyptus* log, 10.iii.1957, 2 ad., 1 juv. 11.

HANSENIELLA SIMILIS, sp. nov.

(Fig. 10)

Holotype. One of the adult specimens from Mt. Toolbrunup, Stirling Ranges, W.A., 12.vi.1953.

Length.—Holotype rather contracted, measuring 1·65 mm; remaining adult specimens varying between 2·24 and 3·05 mm.

Head.—Head with the same breadth as length. Lateral angle at point of articulation of mandible marked but rounded. The short but conspicuous central rod visible only in hind half of head. Its length from posterior end-swelling to disappearing point forwards a little smaller than one-quarter of the length of head. No lateral branches have been observed, and in front the rod becomes more and more indistinct and disappears just behind centre of head. Dorsal surface of head densely covered with straight and evenly scattered setae, the longest being a little shorter than three-quarters of the greatest diameter of 1st antennal segment.

Antennae. Antennae broken or lost, but basal part of antennae of holotype suitable for study. Primary whorl of 1st segment comprising 6 setae, two of which located on dorsal side, 2 on inner, and 2 on ventral side. At least 4 of these setae inserted on inner half of the segment. Between proximal end of 1st segment and primary whorl there is a shorter seta inserted on upper part of inner side. Second segment too with a single primary whorl. Its setae rather evenly scattered around the segment. Setae of inner side of about the same length as the others. The most proximal parts of antennae with only 1 whorl of setae on each segment, secondary whorl beginning on 3rd segment. Small 4-spined organs begin on dorsal side of same segment. All observed antennal segments covered with a delicate pubescence on all sides except the first one where the outer side only has a very sparse pubescence.

Terga.—First tergum rudimentary, bearing 4 setae. Second tergum fully developed with posterior margin convex. It has distinct, lateral angles provided with large anterolateral macrochaetae being directed outwards and forwards.

Between these long setae 21 setae of shorter but different lengths are found, the longest of them being a little shorter than half the length of anterolateral macrochaetae, while the shortest ones being about one-fifth of this length. Surface of tergum set with setae of medium size. This tergum twice as broad as long. Third tergum proportionately longer than 2nd and showing a great resemblance to an equiangular trapezium. Its breadth a little shorter than breadth of head

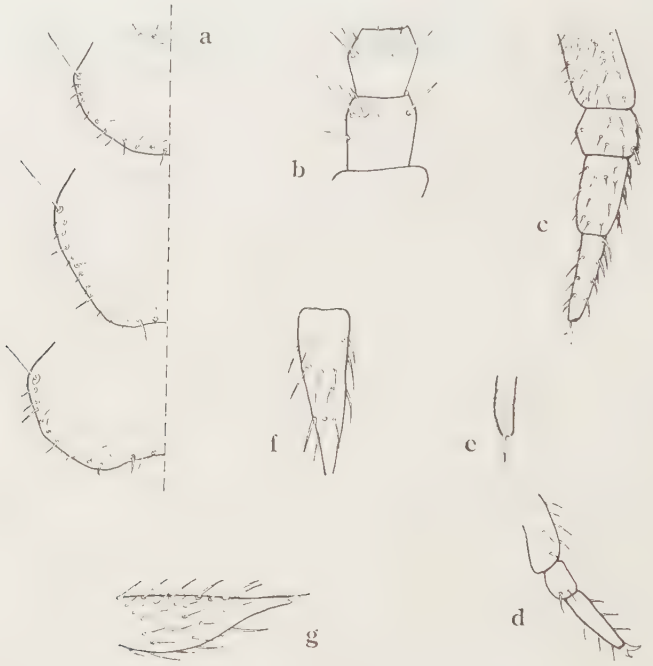

Fig. 10.—*Hanseniella similis*, sp. nov.: *a*, first 4 terga (only marginal setae are drawn); *b*, basal part of right antenna, from above; *c*, 12th leg, from in front; *d*, first leg, from behind; *e*, stylus; *f*, left cercus, from below; *g*, left cercus, from outer side.

and posterior margin only very little indented. There are 27 setae of different lengths between anterolateral macrochaetae. The latter nearly twice as long as diameter of 1st antennal segment. Fourth tergum at least twice as broad as long with posterior margin rather deeply emarginate. Most of the anterolateral macrochaetae are missing behind 4th tergum, but judging from areas of insertion, it seems as if terga 2, 3, 4, 6, 7, and 9 all have such setae.

Legs.—Tarsus of 12th pair of legs fairly slender at distal end, but widens a little towards upper part being a little more than 4 times longer than wide with both dorsal and ventral side straight. This joint covered with numerous setae arranged in rows lengthways. In the dorsal row there are 4 setae and so also in the ventral. All dorsal setae of nearly the same length, the longest being as long as or a little longer than greatest width of the joint. Tibia twice as long as wide and like tarsus covered with setae on all sides, 3 in dorsal and 3 in ventral rows.

The longest of these setae is located on dorsal side near distal end and its length is about as long as the greatest width of the joint. Femur too covered with numerous setae of which the most distal one of dorsal side is the longest.

First pair of legs short with a nearly cylindrical tarsus tapering towards distal end. This joint is 4 times longer than wide and sparsely set with setae. Distally located setae only a little longer than the others, the longest one being as long as the greatest width of the joint. Third joint with dorsal side naked and ventral surface set with some mostly short setae.

First pair of legs with a very elongate anterior claw, only a little curved, nearly one-quarter the length of tarsus. It is about as long as posterior claw. Anterior claw of 12th pair of legs shows a high degree of similarity to that of 1st leg but is longer, one-quarter to one-third the length of tarsus.

Styli well developed, elongate, with a dense pubescence, 3-3·25 times longer than wide, 1·3 times longer than width of tarsus. The 2 apical setae of very different lengths, the longer one half the length of stylus while the shorter is very minute.

Cerci.—Cerci 3 times longer than wide with a moderate number of setae of medium or fairly great length and arranged in 5 main rows. Length of setae increases distally, the longest ones being the most distally located and their length is reaching 1·2 the depth of cercus. The apical setae are missing in all specimens except two paratypes where the shorter one has been studied. Its length is a little more than one-tenth the length of cerci.

Affinities.—This species belongs to the large group of species with anterolateral macrochaetae on the terga 2, 3, 4, 6, 7, and 9 but it is easily distinguishable from the known species especially by the form of the 3rd tergum.

Material examined.—12 specimens.

Distribution.—WESTERN AUSTRALIA: Stirling Ranges, 310 miles S. of Perth, Mt. Toolbrunup, in *Eucalyptus* sp. and *Melaleuca* sp. leaf litter, 12.vi.1953, 9 ad., 1 juv. 10; the same place, in *Eucalyptus* sp. leaf litter and moss, 12.vi.1953, 2 ad.

Conclusions and Discussion

As may be seen, the major part of the species from Bornemissza's collection are entirely new. There are seven members of the Scolopendrellidae, all new, and six of the Scutigerellidae, all new except *Hanseniella agilis*. Apart from these 13 Symphyla only two more are known from Australia, viz. *H. indecisa* reported from Lion Hill, Guildford, Harvey, Brunswick, Boyanup, and Gooseberry Hill (Attems 1911) and Nornalup, near the Frankland R., and Walpole (Scheller 1954), and Tieg's *H. minor* from Belgrave, Marysville, and the Otway Ranges in Victoria.*

It is especially interesting to establish the presence of the Scolopendrellidae in Australia. The absence of these very minute forms from the earlier collections

* The Australian symphylid Tillyard figured (1930, p. 28, fig. 9) and named *Scolopendrella* sp. is erroneous; the legend to the figure and the figure itself shows that the determination is wrong. The figured animal, having well-developed styli and being as long as 6 mm, must be a Scutigerellidae. A more exact determination is impossible as the figure gives no details.

must depend wholly on deficiencies in the collecting methods. Of the different species, the ones belonging to the genus *Symphylella* are most abundant. *S. australe* seems to be common in south-western Australia (Mt. Toolbrunup and Gnangara). Nearly half of the Scolopendrellidae material belongs to this species. Another species, *S. bornemisszai,* is rich in specimens from one locality (Gnangara) but it has not been found elsewhere. The only Symphyla in Bornemissza's collection (except *S. australe*) which has been found in more than one locality is the south-western (Mt. Toolbrunup and Gnangara) and northern (Kimberley) *Scolopendrellopsis eucalyptica.* The occurrence of this symphylid is remarkable. Although many collections have been examined from various parts of the world, only two species of this genus have been reported. One of them *S. microcolpa* (Muhr) is rare but widely distributed in southern Europe and northern Africa. *Scolopendrellopsis* is also mentioned from California, where Hilton described *S. sensiferis* in 1931, but this species has not been found again anywhere. Thus a general feature of the occurrence of the known Scolopendrellidae in Australia is that the genera have very large areas in the present times while the species seem to be endemic.

If we consider the Scutigerellidae from the same point of view, it is evident that *Hanseniella* is nearly as widely distributed as the Scolopendrellidae genera. It has its chief areas in the southern hemisphere, where it is very widespread and rich in species. However, all its species in Australia except one are confined to this continent. Concerning *Scolopendrelloides,* of which only one species has been found, we know very little about its distributional area (p. 158).

As might be expected, the genera of Symphyla seem to be very old in Australia and the evolution of new types must have advanced slowly, as the genera are common to those on the other continents. However, all genera (except *Hanseniella*) according to the present state of knowledge are composed of endemic species only. The Symphyla here are not only conservative, they are also very uniform in body structure and to some extent the chaetotaxy within the genera shows a high degree of resemblance between the species. This is valid for some *Symphylella* and *Hanseniella* species.

Bornemissza's dispatch list gave many data of the biotopes of the localities, but it is not yet possible to use this information for ecological discussions, as two quite different collecting methods have been applied. Moreover, the number of species is very large in comparison with the number of localities and as stated in the Introduction the localities are dispersed nearly throughout the whole continent. Thus it is not possible to draw any reliable conclusions concerning the origin of the Australian Symphyla or to give any certain indications of the real distribution of different genera and species on the basis of our present knowledge. Future collections may yield a vast material of new forms from most part of the country. However, what we now know will constitute an important basis not only for further investigation of the occurrence of the genera and species but for the establishment of reliable limits of the geographic distribution and for the study of the selective operation of some habitat requirements on the composition of the fauna.

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List of Genera and Species